MULTIPLE MEMORY SYSTEMS AND EXTINCTION:

THE NEUROBIOLOGICAL BASIS OF LATENT EXTINCTION

A Dissertation

by

AMANDA GABRIELE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Psychology

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Approved by

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ABSTRACT

Multiple Memory Systems and Extinction:

The Neurobiological Basis of Latent Extinction. (May 2008)

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Understanding the neural mechanisms underlying the extinction of maladaptive behaviors has become increasingly relevant. Extinction, or the reduction of a response due to lack of reinforcement, is believed to be "new learning." Most extinction paradigms involve the performance of the previously reinforced response in the absence of reinforcement in order for extinction to occur. Conversely, latent extinction is a cognitive form of learning in which the previously rewarded response is *not* made during extinction training. However, until now the neurobiological basis of latent extinction has remained unknown.

This dissertation has three aims to examine the neurobiological basis of latent extinction. Previous research has shown latent extinction to be impaired following hippocampal inactivation and the goal of Aim 1 was to examine other neural systems potentially involved in latent extinction through examination of brain structures such as the dorsal striatum, medial prefrontal cortex, and basolateral amygdala. Additionally, the neurochemical basis of latent extinction is unidentified; therefore Aim 2 addressed this question, specifically investigating the glutamatergic system through both NMDA

receptor agonism and antagonism. Finally, understanding latent extinction may be useful for the extinction of drug addiction. Aim 3 was to examine some clinical implications for the extinction of drug addiction utilizing latent extinction following maze running for an oral cocaine reward.

Reversible neural inactivation studies using the sodium channel blocker bupivacaine demonstrated a selective impairment of response extinction following dorsal striatum inactivation, but no effect on either latent or response extinction following medial prefrontal cortex or basolateral amygdala inactivation. These results, coupled with previous data from our lab demonstrate a double dissociation for extinction behavior. Further, peripheral NMDA receptor agonism with D-cyloserine enhances latent extinction and intra-hippocampal NMDA receptor antagonism with AP5 impairs latent extinction, identifying a role for the glutamatergic system in latent extinction. Finally, oral cocaine administration during acquisition selectively impairs latent extinction indicating that drug use affects the relive use of multiple memory systems during extinction. Overall, the multiple memory systems theory and latent extinction provide a framework with which to further understand the neural mechanisms of extinction behavior.

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INTRODUCTION

Extinction Is "New Learning"

Understanding the neural mechanisms underlying the extinction of maladaptive behaviors such as drug addiction has become increasingly relevant. Extinction, or the reduction of a response due to lack of reinforcement, is also believed to be "new learning" about a previously acquired association (Bouton & Swartzentruber, 1991; Bouton, 2002; 2004). Extinction is characterized by the reduction of a response due to lack of reinforcement. For example, once a tone (conditioned stimulus) has been paired with a shock (unconditioned stimulus), then the animal learns to freeze in response to the tone (conditioned response). When the tone is no longer followed by the shock, the animal will no longer freeze in response to the tone and the conditioned response has been extinguished. There are many theories as to why this reduction in responding happens, primarily whether the original association is degraded ("forgetting") (Rescorla & Wagner, 1972; McClelland & Rumelhart, 1985) or is a new association formed in addition to the original association ("new learning") (Bouton & Swartzentruber, 1991; Bouton 2002; 2004). Despite Pavlov's initial observation that, based on the spontaneous recovery, extinction cannot be regarded as "an irreparable destruction of the conditioned reflex" (1927) , initially it was believed that extinction involved the weakening of the association between the CS and the US and "forgetting" occurred (Rescorla & Wagner, 1972; McClelland & Rumelhart, 1985). From an evolutionary standpoint however, it is much more beneficial for an animal to amend previously acquired learning when it

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This dissertation follows the style and format of *Behavioral Neuroscience*.

receives new information rather than forget the initial learning all together. Bouton and colleagues (Bouton & Swartzentruber, 1991; for review see Bouton, 2002; 2004) have proposed instead that extinction is "new learning" and that the original association between the CS and the US is not degraded based on relapse mechanisms of renewal, spontaneous recovery, rapid reacquisition, and reinstatement that demonstrate the post-extinction return of conditioned responding. These relapse mechanisms are both evidence that the original association remains intact during extinction and that extinction is heavily context dependent.

The *renewal* effect occurs when the context is changed after extinction has occurred and the original conditioned response returns. The most common example is "ABA renewal" in which the original conditioning is performed in context A and extinction is performed in context B. When the animal is again placed in context A, the conditioned response returns. There is also "ABC renewal" and to a lesser extent "AAB renewal." The return of the initial conditioned response clearly illustrates that the original association remains intact. The renewal effect also demonstrates that extinction is context dependent, more so than initial conditioning. While initial conditioning is transferable to a new context (e.g. "ABC" and "AAB" renewal), extinction is not.

The *spontaneous recovery* effect occurs when a significant amount of time passes after extinction training and conditioned responding returns. Again, this would not be possible if the original association was degraded. Bouton argues that spontaneous recovery is further evidence that extinction is context dependent in that extinction also

occurs in a "temporal context," and when the subject is tested outside of the temporal context of extinction, the original conditioned responding returns.

The third relapse mechanism of *rapid reacquisition* occurs when CS-US presentations are reintroduced after extinction has occurred. It is called rapid reacquisition because the conditioned responding returns more quickly than responding to a novel CS. While this effect is not always seen, its presence following certain types of conditioning indicates that the original learning was not degraded since the learning following extinction is acquired more rapidly than if a new association were being made.

The last relapse mechanism is *reinstatement.* Reinstatement occurs when, after the completion of extinction training, a single presentation of the US alone is enough to cause the return of conditioned responding to the CS. Again, this relapse mechanism would not occur if the original association had been degraded. If the original pairing between the CS and the US had been degraded then a single presentation of the US would not be sufficient to signal the animal to respond to the CS again. These four relapse mechanisms of renewal, spontaneous recovery, rapid reacquisition, and reinstatement all indicate that the learning that occurs during extinction is new learning about the original association rather than a degrading or unlearning of the original association. Bouton also proposes that since extinction is particularly sensitive to the context under which it is performed, extinction may involve the placement of the CS in a contextually modulated inhibitory association (for review see Bouton, 2002; 2004).

For the CS to be held in an inhibitory association during the context of extinction and the excitatory association between the CS and US acquired during initial conditioning to remain intact, the CS must be capable of containing both an excitatory

and inhibitory association simultaneously. Tait and Saladin (1986) demonstrated with backward conditioning that a single CS concurrently had an excitatory association with a shock, which produced conditioned lick suppression, and an inhibitory association with the shock, which produced slowed eyeblink conditioning. This experiment provides evidence that an inhibitory association can form after an excitatory association has already been made. Further, since in this case the inhibitory association did not lead to a decrement in the excitatory association, it is possible for the CS to have both an excitatory and an inhibitory association with a single US, supporting the theory that extinction places the CS in an inhibitory association and that the original association is not degraded.

Additional evidence for the "new learning" theory of extinction is provided by the finding that drugs which enhance learning also enhance extinction. If extinction involved the weakening or "forgetting" of initial learning then drugs that enhance learning should certainly not accelerate this process. For example, when glucose or oxotremorine (a muscarinic receptor agonist) is given either systemically or intra-amygdala it will, in much the same manner than it affects learning, facilitate consolidation of conditioned place preference extinction (Schroeder & Packard, 2003; Schroeder & Packard, 2004). Similar results have indicated that molecular mechanisms involved in acquisition and consolidation of contextual fear conditioning are also involved in extinction (Szapiro et al., 2003).

Latent Extinction: Cognitive or Stimulus-Response

Most extinction paradigms involve the performance of the previously reinforced response in the absence of reinforcement, which will be termed "response extinction."

For example, if an animal has been trained to barpress for a food reward, extinction of the barpress response requires responding in the absence of reinforcement in order for the animal to learn that barpressing no longer leads to food reward. However, it has been demonstrated that in some learning situations, extinction is possible *without* the explicit performance of the previously reinforced response in the form of latent extinction. The phenomenon of latent extinction was discovered in 1949 (Seward & Levy, 1949) and was based on Tolman's theory that animals can acquire learned expectancies, (i.e. the idea that they learn "what leads to what" (Tolman, 1932). According to this theory, if an animal is trained to run to a goal box for food reward and subsequently finds the goal box empty, it only needs to recall its emptiness for extinction to be possible. This "cognitive" version of extinction was in direct contrast to stimulus-response theory which states that behavior is guided based on chains of associations between stimuli and responses. These associations are strengthened following reinforcement, but the reinforcement is not part of the association and the animal does not acquire expectancies about its behavior (Hull, 1943). Interestingly, O'Keefe and Nadel refer to latent extinction is a "pure" form of cognitive extinction (p. 342, 1978) In the original demonstration of latent extinction, rats were trained to locate a food reward on an open straight alley maze. An experimental group was given "pre-extinction" placement trials in the goal platform with no reward present (latent extinction) while a control group received pre-extinction placements on a neutral platform located six feet away from the maze. When tested under normal "response" extinction trials (i.e. maze running with no reward present) the experimental group showed longer latencies to reach the goal box as compared to controls. Additionally, the experimental group required fewer trials to reach the extinction criterion

of two successive refusals to exit the start box (Seward & Levy, 1949). Seward and Levy interpreted these findings as evidence that reward expectancy and goal anticipation play a significant role in maze learning (1949). An analogous study questioned whether a discrimination response on a U-maze could be extinguished without performance of the choice point response. Similar to the results obtained by Seward and Levy, placements in the previously rewarded goal box were sufficient to extinguish responding. Additionally, extinguished responding following latent extinction did not display spontaneous recovery 24 hours after the initial extinction test. The authors concluded that this study provides evidence that Hull's "response-induced inhibition" (1943) theory of extinction is insufficient to explain latent extinction. According to this theory, un-reinforced responses result in an inhibitory state and that this inhibitory state replaces the positive response associated with the stimuli present, therefore inhibiting responding. The phenomena of latent extinction proved to be difficult for S-R theory to explain since the conditioned response was not explicitly performed during extinction, therefore not allowing for the "inhibitory state" to occur during extinction (Deese, 1951).

However, Bugelski et al. (1952) indicated that the experimental and control groups differed on *two* variables: the location of pre-extinction confinement and the amount of time spent on the goal platform during traditional extinction trials. In Seward and Levy's (1949) original investigation, the experimental group received extended goal platform placements both prior to traditional extinction trials and in between trials (120s) whereas the control group only received brief (20s) confinement in the goal platform during extinction trials and then removed to the neutral platform for the remaining intertrial interval (100s). When Bugelski et al. (1952) replicated the 1949 study and

included a control group that received pre-extinction trials on a neutral platform but also received a extended placements (120s) on the goal platform in between extinction trials, the enhancement effect of pre-extinction exposures in the goal box as compared to this new control group disappeared. However, they also failed to replicate the robust effect demonstrated previously by Seward & Levy in the experimental group (Bugelski et al., 1952). This apparent discrepancy was investigated by Denny & Ratner (1959), who determined that a potential difference between the two studies was the extra-maze environment. Bugelski et al. (1952) lacked distinctive extra-maze stimuli at the goal box and when this omission was rectified, the latent extinction enhancement effect was observed (Denny & Ratner, 1959). Although latent pre-extinction exposures have clearly been demonstrated to cause an enhancement of extinction **(**Seward & Levy, 1949; Deese, 1951; Denny & Ratner, 1959; Motlz, 1955; Moltz & Maddi, 1956), the learning that underlies latent extinction was still under question.

Since Hull's extinction theory was unable to explain the latent extinction effect, S-R theorists sought to provide an alternate S-R theoretical explanation, employing the "fractional anticipatory response" mechanism (Moltz, 1957). It is important to note that the fractional anticipatory response is viewed by some as a "*dues ex machina* for non-expectancy theorists" (Meehl & MacCorquodale, 1951). Moltz (1957) proposed that during maze acquisition training, when the full consummatory response (R_G) is reinforced, it becomes conditioned to goalbox stimuli. These goalbox stimuli will then generate all parts of R_G that are not incompatible with other responses (e.g. the fractional anticipatory goal response or r_G). An apparent example of r_G is rats running to the right of the maze runway in a T-maze when the goal was on the right arm, and to the left of the

runway when the goal was on the left arm (Miller, 1935). Thus, the fractional anticipatory response, or r_G, is evoked repeatedly and becomes conditioned to stimuli in the goal as well. Therefore, during extinction (including latent extinction), r_G is elicited by these goalbox stimuli but is not reinforced, and this nonreinforcement leads to a reduction in response strength. If the stimuli in the goalbox are similar to the stimuli present in the rest of the maze, the extinction of r_G should generalize and lead to increased latencies as seen following latent extinction (Moltz, 1957). Moltz supported these arguments with two studies examining latent extinction as related to secondary reward value. In this case, goalbox cues such as a foodcup that are closely associated with reinforcement develop reinforcing properties themselves, or secondary reward value, and therefore have the power to stimulate responses associated with the reward (e.g. r_G). In the first experiment, which is a form of "Type I latent extinction" (Moltz, 1957), animals were trained in an enclosed straight alley maze with a curtain around it to minimize exposure to extra-maze cues. Two experimental groups received latent extinction placements in the goalbox, with the white painted foodcup used during acquisition present for one group and absent for the other. A third control group did not receive latent extinction placements. Moltz (1955) predicted that for the group with the foodcup present during extinction, the secondary reward associated with the foodcup would diminish and the latent extinction effect would be seen regardless of whether the foodcup was present or absent during traditional response extinction trials. However, for the group with the foodcup absent during extinction, the foodcup would retain its secondary reward value, and therefore replacing the foodcup during traditional response extinction trials would cause responding to be maintained. The results supported these

predictions, allowing Moltz to conclude that latent extinction involves the reduction of the secondary reward value of goalbox cues (1955). In a second experiment, examining what Moltz (1957) terms "Type II latent extinction," animals were trained and given latent extinction in a straight alley maze, but the goal box was removed and placed on a T-maze for extinction assessment. Two distinct goalboxes were used, one consistently reinforced and one consistently non-reinforced. An additional variable of hunger drive was also introduced during the latent extinction period. Animals that received latent extinction training in the straight alley showed a significant latent extinction effect as characterized by fewer correct responses in the T-maze than those that did not receive latent extinction training. Additionally, within the latent extinction group, animals with a high hunger drive (44 hrs. deprived) showed fewer correct responses than those with both a lower hunger drive (22 hrs. deprived) or no hunger drive (0 hrs. deprived). Moltz took these findings as further evidence that the goalbox cues contain secondary reward value since the latent extinction effect was transferable to another maze, therefore mediating a new response. Additionally, it was predicted that a higher hunger drive would lead to more evocations of r_G during latent extinction. Since according to Moltz the greater number of non-reinforced evocations of r_G, the greater the reduction in responding, a higher hunger drive should lead to more significant reductions in responding, as demonstrated by the data (Moltz & Maddi, 1956). While Moltz took this data to signify the secondary reward value of the goalbox cues and thus support for his S-R theory of latent extinction, other considerations must be made. In the experiment examining "Type I latent extinction," a significant effort was made to eliminate extra-maze cues thereby limiting any potential cues to be associated with reward to the foodcup. However, an

expectancy theory cannot be ruled out. During acquisition, the animal could learn that the foodcup predicts reward, especially given the lack of other contextual cues with potential predictive value. If the foodcup is present during latent extinction, then the association is made that the foodcup no longer predicts reward. However, if the foodcup is absent during latent extinction, its positive prediction of reward remains the same, therefore reintroducing the foodcup during the extinction test should reintroduce responding. A similar explanation can be made for "Type II latent extinction." If the animal had learned that a previously reinforced goalbox no longer predicts reward then it is expected that placing this goalbox on a new maze would not induce responding. Since both S-R theory and expectancy theory predict the same outcome of the previously described experiments, it is impossible to view the results as confirmation of an S-R explanation of latent extinction. In a similar study, animals were trained on a T-maze to run to a goalbox containing a glass foodcup dish and a pellet feeder tube, which made a loud clicking noise as each pellet was dispensed. During latent extinction animals either received confinement in the goalbox described above, or in a goalbox without the dish, dispenser tube, or clicking sound. It was predicted, per S-R theory, that increasing the number of goalbox stimuli would have the result of increasing evocations of r_G, and therefore a stronger latent extinction effect. However, a stronger latent extinction effect of longer latencies and fewer correct arm choices was seen in the group that received latent extinction *without* the goalbox stimuli present. While the authors argued that the not yet extinguished secondary reinforcing property of the "click" sound maintained responding (Chapman & Carlson, 1963), again an explanation involving expectancy theory can be made. Since during acquisition the goalbox contained several cues that strongly predicted

food reward, removing those cues made the lack of reward in the goalbox more salient, allowing the animals to more easily associate the empty goalbox with non-reward.

Two points made by the fractional anticipatory response theory of latent extinction are important to note; that reduction in response strength relies on repeated non-reinforced elicitations of r_G during latent extinction, and that this theory depends upon the ability of r_G to generalize to the rest of the maze (Moltz, 1957). Therefore it would be predicted that a) the more frequently r_G is elicited in the goalbox, the stronger the reduction in responding and b) a high degree of similarity between the goalbox and the rest of the maze would lead to a more robust latent extinction effect. Several experiments were conducted in an attempt to support these predictions and provide further verification that latent extinction could be explained in S-R terms.

Studies that examined food cup responses as a measure of fractional anticipatory responses demonstrated that during pre-extinction goalbox placements in a T-maze, those animals with more food cup responses showed faster latencies and more correct arm choices (Thomas, 1958; Chapman & Carlson, 1963). These findings are in direct contrast with Moltz who predicted that "the more frequently r_G is evoked during latent extinction the greater will be the reduction in its response strength" (Moltz, 1957 pg. 236). Thomas (1958) explains this discrepancy by concluding that persistent food cup responses during extinction indicate that the secondary reward value of the food cup has not diminished sufficiently to lead to a decrement in responding (Thomas, 1958). However, it seems unlikely that increasing food cup responses (i.e. more frequent evocations of r_G) should lead to both response perseveration if the association has not been extinguished and a decrease in responding if the association has been extinguished.

Additional studies examined the prediction that similarity between maze and goalbox cues should result in a more robust latent extinction effect. However, animals trained and extinguished with a distinctive goalbox showed a more significant latent extinction effect as compared to both animals trained and extinguished with a non-distinctive goalbox (an extension of the runway) and controls not receiving latent extinction placements (Hughes et al., 1960). A follow up study from the same lab aiming to eliminate an apparent discrepancy between the sizes of the goalboxes in the previous study obtained similar findings (Koppman & Grice, 1963), further demonstrating that, contrary to the predictions of S-R theory, a more distinctive goalbox produces a *stronger* latent extinction effect. An explanation more consistent with expectancy theory is that a distinctive goalbox would more readily allow the animal to associate that particular location with non-reward. However, further attempts were made to use stimulus-generalization to explain latent extinction. In the experiments described above, runs to the reinforced and non-reinforced arms were equated through a forcing procedure allowing for equivalent exposure to each goalbox. An argument can be made that, in the case of the animals receiving reinforcement in the distinctive goalbox, non-reinforced exposure to the non-distinctive goalbox increased the association of non-reinforcement in the runway itself due to the high degree of similarity between the non-distinctive goalbox and the runway. Therefore during the test trials, decreased responding in the runway seen in the distinctive goalbox group may be the result of the effects of an association of non-reinforcement in the runway rather than a distinct latent extinction effect (Patten & Hendricks, 1971). This explanation seems unlikely since this effect should have been readily apparent in acquisition as well. Especially given that if an association between the

runway and non-reinforcement leads to decreased responding, an association between the runway and reinforcement (as would be predicted in the group that received reward in the non-distinctive goalbox) should lead to increased responding. However, in order to investigate these claims, an experiment was conducted in the absence of runway responding during acquisition. During acquisition, animals received rewarded latent placements in the goalbox of a straight runway that was an extension of the maze (non-distinctive). Animals that received latent extinction placements in the maze goalbox used in training showed higher latencies at the test trial as compared to animals that did not receive latent extinction. However, animals that received latent extinction placements in a new distinctive goalbox did not show a latent extinction effect and had similar latencies at the test trial to animals that did not receive latent extinction placements. The authors took this data to demonstrate the role stimulus-generalization in latent extinction (Patten & Hendricks, 1971). However, expectancy theory can again be applied for an alternate explanation. The animals receiving latent extinction in the distinctive goalbox received two discrete contexts in one spatial location, one that predicted reward and one that predicted non-reward. Upon being places in the startbox of the runway, having not received previous experience in that portion of the runway, it is possible that the animals did *not* know "what leads to what" in the sense that they are unable to predict which goalbox would be at the end of the maze, particularly since the extinction test consisted of a single trial. Further, since extinction is extremely context dependent (Bouton, 2002; 2004), the startbox could potentially have been viewed as a third maze context and responding was stimulated as seen in ABC renewal. Interestingly, it has been demonstrated that when animals are trained on two different runways to two different

goalboxes occupying the same spatial location, and given latent extinction placements in one goalbox and latent rewarded placements in the other, they are able to discriminate between the goalboxes during test trials (Gaffan & Gowling, 1984).

Additionally, a further inconsistency in the predictions of S-R theory was noted by Dyal (1962). According to the fractional anticipatory response theory, once the animal has completed the latent extinction and proceeds to regular extinction trials, the animal should continually increase running times since further un-reinforced presentations of the goalbox cues should strengthen extinction. It was found that this is not the case. By the end of five regular extinction trials, the difference in latencies between animals that received latent extinction and controls disappears (Dyal, 1962). In general, the predictions made by S-R theory as an explanation for latent extinction have not been verified by experimental data.

In addition to the experimental evidence described above, several theoretical explanations have been made that demonstrates the insufficiency of S-R theory and the fractional anticipatory response mechanism to explain latent extinction (Gleitman et al., 1954; Treisman, 1960). Gleitman et al. (1954) contend that since r_G is an implicit response, requiring little effort, a significant amount of trials should be required for extinction. Since studies demonstrating latent extinction have given relatively few placements in the goal box (e.g. 4 or 5, Deese, 1951; Seward & Levy, 1949 respectively), this does not seem to be the case. However, the argument can be made that this line of reasoning does not account for the ability of the goalbox to elicit r_G "freely and repeatedly" (Moltz, 1957). A more compelling argument was presented by Treisman (1960). When the fractional anticipatory response (r_G) is performed in the goal box it

produces a fractional goal stimulus (s_G), which is part of the full proprioceptive stimulation (S_G) produced by the full consummatory response (R_G). Since this r_G- s_G association coincides with reinforcement s_G, according to S-R theory, develops secondary reinforcing power. Secondary reinforcement occurs when a neutral stimulus corresponds with a reinforcing state, which causes the stimulus itself to develop reinforcing properties. According to Moltz, once r_G is no longer followed by reinforcement, as during latent extinction, the subsequent associations r_G- s_G will weaken. However, Treisman (1960) argues that due to the secondary reinforcing properties of s_G, r_G is always followed by a reinforcing state therefore strengthening this association. Further, since Moltz uses the secondary reinforcing power of s_G to explain the reinforcement of r_G in other areas of the maze as a result of stimulus generalization, it cannot be argued that s_G is not strong enough to maintain associations on its own. Therefore, r_G- s_G associations should be maintained during latent extinction and thus maintain responding. Additionally, since s_G is a part of the full consummatory response, it should be reinforced whenever the animal eats, on the maze or not (Treisman, 1960). Unintentional support for this theory has been provided with the demonstration that a cue with supposed high secondary reinforcing power (feeder click) presented during latent extinction actually sustained responding at the test trial (Chapman & Carlson, 1963), rather having a facilitory effect over extinction as the fractional anticipatory response theory would predict. This argument utilizes S-R theory to demonstrate that the fractional anticipatory response mechanism, cannot satisfactorily explain latent extinction. While significant work has been done to examine variables that effect latent extinction, the neurobiological basis of latent extinction has remained largely unknown.

Multiple Memory Systems and Extinction

According to multiple memory systems theory, learning is organized in separate and dissociable neural systems that mediate different types of memory. The hippocampus mediates cognitive or relational memory while the dorsal striatum mediates habit based stimulus-response learning in both animals (e.g., Cohen & Squire, 1980; Eichenbaum & Cohen, 2001; Hirsh, 1974; Mishkin & Petri, 1984; O'Keefe & Nadel, 1978; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002; Zola-Morgan & Squire, 1984) and humans (Scoville & Milner, 1957; Knowlton, et al., 1996). Until now multiple memory systems research has focused on the initial acquisition of learned behavior, however research from our lab has indicated that multiple memory systems are also involved in extinction behavior. Latent extinction, as a cognitive form of extinction, provides itself as a useful tool for applying the multiple memory systems theory to extinction since it is readily dissociated from the habitual runway responding as seen in traditional extinction paradigms. Reversible inactivation of the dorsal hippocampus impairs latent extinction while response extinction remains intact. This dissociation indicates that this cognitive form of extinction is hippocampus-dependent (Gabriele & Packard, 2006). Dissociation methodology is the most effective way to demonstrate functional independence to support the multiple memory systems theory. A double dissociation would illustrate that a lesion in brain area A impairs task C and not D, and that a lesion in brain area B impairs task D and not C. Following a multiple memory systems hypothesis of extinction, similar to initial task acquisition the hippocampus mediates latent extinction and it is suggested that the dorsal striatum mediates stimulus-response extinction (Gabriele & Packard, 2006).

Goals of the Current Research

This dissertation has three aims that examine the neurobiological basis of latent extinction. Previous research has shown latent extinction to be impaired following hippocampal inactivation (Gabriele & Packard, 2006) and the goal of the first aim is to examine other neural systems potentially involved in latent extinction. Since the dorsal striatum mediates initial acquisition of habit based S-R learning and not "cognitive" relational learning (Graybiel, 1998; McDonald & White, 1993; Packard & Knowlton, 2002; Knowlton et al., 1996; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002), Experiment 1 aimed to examine whether the dorsal striatum is required for latent extinction and also to examine whether the dorsal striatum plays a role in response extinction. Experiments 2 & 3 examined other brain structures implicated in extinction such as the medial prefrontal cortex (Morgan et al., 2003; Hugues et al., 2004; Santini et al., 2004; Sierra-Mercado et al., 2006; Sotres-Bayon et al., 2004) and the basolateral amygdala (Falls et al., 1992; Walker et al., 2002; Schroeder & Packard, 2003; 2004) to determine if they contribute to the acquisition of latent extinction.

Although we have shown latent extinction is hippocampal dependent (Gabriele & Packard, 2006), the neurochemical basis of this behavior unknown. The second aim was to examine the neurochemical basis of latent extinction, specifically whether the glutamatergic system, a neurotransmitter system implicated in fear extinction (for review see Myers & Davis, 2007), is also involved in latent extinction. Experiment 4 investigated whether peripheral administration of an NMDA agonist, D-cycloserine, which has been shown to enhance other types of extinction (for review see Richardson et al., 2004; Ressler et al., 2004; Hofmann et al., 2006), also enhances latent extinction.

Experiment 5 addressed whether intra-hippocampal administration of an NMDA antagonist, AP-5, impairs latent extinction. Further, Experiment 5 examined whether the type of learning used to acquire a task can influence the type of learning that can be used to extinguish that task.

Finally, since the extinction of maladaptive behaviors is extremely clinically relevant, understanding latent extinction may be useful for clinical therapies targeting drug addiction. Latent extinction, as a "cognitive" form of extinction, can be used to address the impact of drug use on various types of extinction therapies. The third aim examined some clinical implications for the extinction of drug addiction utilizing latent extinction. Experiment 6 addressed this aim by examining whether oral cocaine administration influences the effectiveness of latent extinction.

CHAPTER II

GENERAL METHOD

Subjects

Subjects were adult male Long-Evans rats (275-300 g). Rats were individually housed on a 12:12 hour light-dark cycle, with lights on from 8:00 a.m. to 8:00 p.m. All animals received food and water *ad libitum* prior to behavioral testing.

Apparatus

Straight Alley Maze

For straight alley experiments, the apparatus was an elevated (34 inches) straight alley maze with a black Plexiglas floor and clear Plexiglas sides (70 in. long, 4.5 in. wide and 8 in. tall). A food cup (1 inch diameter) was located at the goal end of the maze. The maze was located in a room containing several extra-maze cues. A schematic of the straight alley maze is located in Appendix A.

Water Plus-Maze

For water plus-maze experiments, a clear Plexiglas plus-maze (43 cm height, arm-width of 27 cm, and arm-length of 60 cm) was inserted in a black circular water maze (180 cm diameter, 45 cm height). The water maze was filled to a water level of 21 cm and temperature was maintained at 25ºC. An invisible clear Plexiglas escape platform (15 x 14 x 20 cm) was placed inside of the plus maze at the end of the designated goal arm, 1 cm below water level. The arm opposite to the start arm was blocked by a piece of clear Plexiglas so that the animals were trained in a T-maze configuration. The maze was located in a room containing several extra-maze cues. A schematic of the water plus mazes is located in Appendix A.

Surgery

Rats were anesthetized with isoflurane gas anesthesia (Vedco) and bilateral guide cannula (23 gauge, 10 or 15 mm long) were inserted into either the dorsal hippocampus, dorsolateral caudate, basolateral amygdala, or prefrontal cortex using standard stereotaxic techniques. Coordinates for the dorsal hippocampus were anterior-posterior (AP) = -3.1 mm from bregma, medial-lateral (ML) = +/- 2.0 mm, and dorsal-ventral (DV) = -2.0 mm from skull surface; dorsolateral caudate AP = -0.3, ML = +/- 4.2, DV = -4.0; basolateral amygdala AP = -2.2, ML = +/- 4.7, DV = -7.0; and medial prefrontal cortex AP = +3.2, ML = +/- 0.75, DV = -3.5. Animals were allowed to recover for one week following surgery.

Infusions

Bilateral brain infusions (0.5 µl/side) were administered using an electronically timed microsyringe pump (Sage Instruments) and10 µl Hamilton syringes connected to injection needles (11 or 16 mm length, 30 gauge) via polyethylene tubing (PE 10). Infusions were administered over a period of 54 seconds, and the injection needles were left in the guide cannula for an additional 60 seconds to allow for diffusion. This infusion procedure was identical to that of our previous study indicating a role for the dorsal hippocampus in latent extinction in a runway (Gabriele & Packard, 2006).

Histology

Following the completion of behavioral procedures, rats were anesthetized with 1 cc of sodium pentobarbital (60 mg/kg) and perfused with physiological saline followed by a 10% formal-saline solution. Brains were removed and sectioned via cryostat at 20

um and stained with cresyl violet. Cannula locations were verified using a standard rat brain atlas (Paxinos and Watson, 1986). Histology figures can be viewed in Appendix B.

Statistics

A two-way one-repeated measures ANOVA was used to analyze running latencies during initial training to determine that are no differences in groups prior to extinction training in terms of acquisition. Probe trial latencies were analyzed using a one-way ANOVA.

CHAPTER III

EXPERIMENT 1

Introduction

Initial acquisition of S-R habit learning is mediated by the dorsal striatum (Graybiel, 1998; McDonald & White, 1993; Packard & Knowlton, 2002; Knowlton et al., 1996; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002). In view of evidence that extinction is new learning (Bouton & Swartzentruber, 1991; Bouton, 2002; 2004) and that the dorsal striatum plays a significant role in the acquisition of stimulus-response habits, it is possible that this structure may also play a role in the extinction of habit learning.

Studies examining acquisition of learned behaviors have dissociated hippocampus dependent cognitive/relational learning from dorsal striatal dependent stimulus-response habits and have indicated that an intact striatum is not necessary for the acquisition of cognitive memory (McDonald & White, 1993; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002). Acquisition of the straight alley task may involve developing both a habitual running response and a cognitive association of the goalbox location with reward so presumably extinguishing either one of those associations should lead to a decrement in runway responding behavior. According to a multiple memory systems hypothesis of extinction, the dorsal striatum should be responsible for the acquisition of response extinction but not required for hippocampus-dependent "cognitive" latent extinction (Gabriele & Packard, 2006). Response extinction is a more traditional extinction paradigm containing the S-R component of involving the explicit extinction of the habitual running response; therefore the dorsal striatum may contribute

to the acquisition of this form of extinction. This form of extinction readily fits with Hull's response inhibition theory of extinction (1943).

Dorsal striatum lesions have been demonstrated to impair initial acquisition of straight alley runway behavior (Kirkby, Polgar, & Coyle, 1981) and while there have been limited studies examining the function of the dorsal striatum in extinction, a few studies have investigated extinction of the runway task. Following initial acquisition in a runway task, the ventrolateral caudate was lesioned with kainic acid and acquisition was resumed. Animals were then trained with a go/no go paradigm in which only the odd trials were reinforced. Ventrolateral caudate lesions only led to impairments in extinction, however all groups were unable to acquire the go/no go version of the task (Dunnett & Iversen, 1981). Similarly, Thullier et al. (1996) found that pre-training electrolytic lesions of the caudate putamen impaired extinction of a traditional runway task further indicating perseverative responding, likely due to impairments in the habit memory systems. The current study utilized reversible lesions conducted immediately pre-extinction to more clearly elucidate the function of the dorsal striatum in runway extinction.

Within the dorsal striatum, the lateral region may be more selectively involved in the formation of stimulus-response habits (Yin & Knowlton, 2004; Yin et al., 2004). Further, the lateral region of the dorsal striatum contains several functionally dissociable regions based on cortical projections of different types of sensory information. For example, the posterio-ventrolateral caudate receives visual input and is therefore involved in procedural learning based on visual discrimination (Viaud & White, 1989). The dorsolateral caudate receives vestibular/kinesthetic input from the somatosensory cortex and has been implicated in body-turn mediated response learning in the plus maze

(Packard & McGaugh, 1996). The dorsolateral caudate was chosen as the target region for the present experiments due to the vestibular/ kinesthetic input provided from maze running that may contribute to the formation of the habitual running response. Additionally, the straight alley maze used in the present experiments was an open maze with no single cue in close approximation to the goal in order to prevent any direct S-R association based on visual cues during acquisition, therefore potentially ruling out the involvement of the posterio-ventrolateral caudate.

Experiment 1 examines the effect of reversible dorsolateral caudate lesions on both latent and response extinction with the predication that caudate inactivation will selectively impairment of response extinction.

Method

Subjects

Subjects were 43 adult male Long-Evans rats (275-300 g). All animals received water *ad libitum*.

Apparatus

The straight alley maze described in the general methods is used for experiment 1.

Surgery

Animals received bilateral cannulation surgeries in the dorsolateral caudate as described in the general methods.

Drugs and Infusions

A 0.75% bupivacaine solution (Abbott Laboratories) was used to produce reversible inactivation of the caudate. Bupivacaine produces a temporary and reversible inactivation of neural tissue via blockade of sodium channels, and hence action potential

conductance. The duration of action of bupivacaine has been estimated at 30-50 minutes (Caterall & Mackie, 1986). Control animals are infused with physiological saline. Bilateral intra-caudate infusions are administered as described in the general methods. This infusion procedure was identical to that of our previous study indicating a role for the dorsal hippocampus in latent extinction (Gabriele & Packard, 2006).

Straight-Alley Maze Acquisition Training

Prior to training, rats were reduced to 85% of *ad lib* body weight and maintained at this weight throughout training. Animals were habituated for one day to the straight alley maze in a single two-minute trial with no food available. Following habituation, rats received 15 Noyes food pellets (Formula P, 45 mg size) in their home cage. On day 1 of food-rewarded training, rats were placed in the start end of the maze and shaped to approach the food cup at the goal end of the maze by placing six pellets along the length of the alley and a single pellet in the food cup. On days 2-10 of food-rewarded maze training (6 trials per day/30 second inter-trial interval), rats were placed in the start end and allowed to traverse the maze and consume one food pellet from the food cup. Upon reaching the food cup and consuming the pellet, rats were removed from the maze and placed in an opaque holding box adjacent to the maze for a 30 second inter-trial interval. If a rat failed to reach the food cup within 60 seconds it was removed for the inter-trial interval. On each trial, the latency (seconds) to reach the food cup was recorded and used as a measure of task acquisition.

Extinction Training: General Procedure

Twenty-four hours following the completion of acquisition training (i.e. day 11), rats were matched based on latencies to reach the food cup during the last three days of

food-rewarded training to form a total of four extinction groups; two "response" extinction groups (bupivacaine, n =10; and saline, n = 9), and two "latent" extinction groups (bupivacaine, n = 12; and saline, n = 12). For both the response and latent conditions, extinction training was administered over three days (6 trials/day, 30 second inter-trial interval), and rats received intra-caudate infusions of saline or bupivacaine immediately prior to extinction training on each of these three days.

Latent Extinction Training

In the latent extinction condition rats were placed by the experimenter facing the empty food cup in the goal end of the maze and were confined for 60 seconds by placement of a clear Plexiglas shield (8 inches from the end of the maze arm). Following confinement, rats were removed from the maze and placed in an opaque holding box located on a table adjacent to the maze for a 30 second inter-trial interval.

Response Extinction Training

In the response extinction condition, rats were placed into the start end of the maze as during training and allowed to run to an empty food cup at the goal end of the maze. Upon reaching the empty food cup (or after 60 seconds if the rat does not reach the food cup), rats were removed from the maze and placed in an opaque holding box located on a table adjacent to the maze for a 30 second inter-trial interval. Latency to reach the food cup was recorded and used as a measure of extinction behavior.

Extinction Testing

On day three of extinction, 90 minutes following the sixth daily extinction trial, all rats were given an additional four extinction "probe" trials in which they were placed in the start end of the maze and latency to reach the empty food cup was recorded. These

four trials allowed for an assessment of the effectiveness of the latent extinction procedure in saline and bupivacaine treated rats. The 90-minute time point for administering these trials was selected based previous work from our lab examining the effect of hippocampal inactivation on latent extinction (Gabriele & Packard, 2006).

Results

Latent Extinction

A one-way ANOVA indicated no difference in latent extinction across probe trials for those animals receiving intra-dorsolateral caudate infusions of bupivacaine (M = 17.896, SEM = 13.558) as compared to controls (M = 18.625, SEM = 15.981) across probe trials ($F_{1,22} = 0.015$, n.s.) (Figures 1.1, 1.2). Further analyses revealed that there was a significant difference between the last day of acquisition training and the probe trials for both animals that received bupivacaine (last acquisition M = 5.843, SEM = 4.495; probe M = 17.896, SEM = 13.558) ($F_{1,22} = 8.545$, $p < 0.005$) and controls (last acquisition M = 6.978, SEM = 7.158; probe M = 18.625, SEM = 15.981) ($F_{1,22} = 5.308$, $p < 0.05$) indicating that both groups displayed a significant extinction effect. Additionally, in a two-way one-repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,22} = 33.409$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for drug treatment ($F_{1,22} = 0.045$, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,22} = 0.162$, n.s.) indicating that both groups acquired the task at a similar rate.

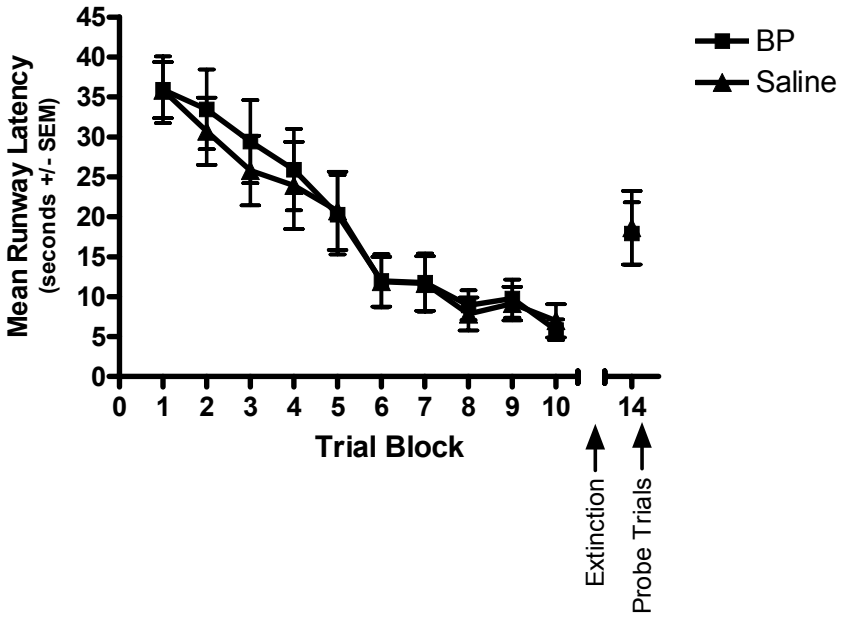

Figure 1.1 The effect of dorsolateral caudate inactivation on latent extinction (experiment 1). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. Dorsolateral caudate inactivation did not affect latent extinction. BP = 0.75% bupivacaine.

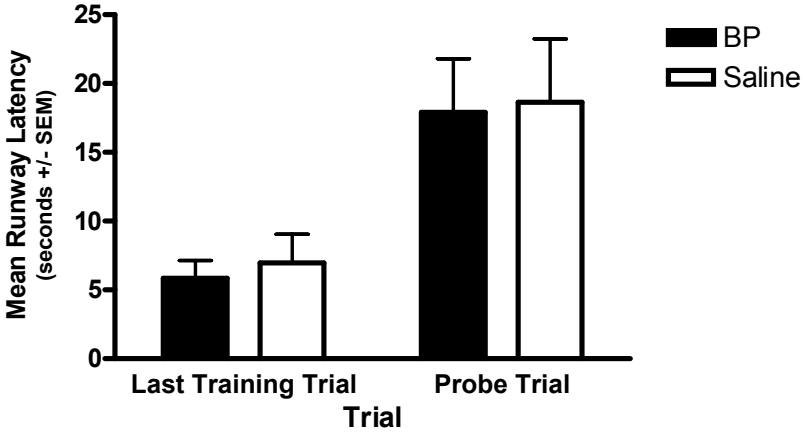

Figure 1.2 Latent extinction following dorsolateral caudate inactivation (experiment 1). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. Dorsolateral caudate inactivation did not affect latent extinction.

Response Extinction

A one-way ANOVA indicated no difference in response extinction across probe trials for those animals receiving intra-dorsolateral caudate infusions of bupivacaine (M = 25.500, SEM = 20.057) as compared to controls (M = 32.917, SEM = 19.046) ($F_{1,17}$ = 0.679, n.s.) (Figures 1.3, 1.4). However, a two-way one-repeated measures ANOVA indicated intra-caudate infusions of bupivacaine selectively impaired response extinction as compared to controls, with a significant main effect for extinction day ($F_{2,17}$ = 4.941, $p < 0.05$), and a significant effect for drug treatment ($F_{1,17}$ = 9.985, $p < 0.01$) but no significant interaction between extinction day and drug treatment ($F_{2,17}$ = 0.075, n.s.) were found, indicating a significant difference across extinction days. Additionally, when the probe trials were included with the three extinction days in a two-way one repeated measures ANOVA, similar results were found of a significant main effect for extinction day ($F_{3,17}$ = 4.008, $p < 0.05$), and a significant effect for drug treatment ($F_{1,17}$ = 5.078, $p < 0.05$) but no significant interaction between extinction day and drug treatment ($F_{3,17}$ = 0.052, n.s.) were found, indicating animals receiving intra-caudate inactivation were generally impaired across all response extinction measures. Also, there was a significant difference between the last day of acquisition training and the probe trails for both animals that received bupivacaine (last acquisition M = 4.284, SEM = 3.122; probe M = 25.500, SEM = 20.057) ($F_{1,18}$ = 10.924, $p < 0.005$) and controls (last acquisition M = 4.956, SEM = 4.025; probe M = 32.917, SEM = 19.046) ($F_{1,16}$ = 18.559 $p < 0.005$) indicating that both groups displayed a significant extinction effect. Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,17}$ = 14.285, $p < 0.001$) indicating significant differences in latencies between days.

There was not a significant main effect for treatment ($F_{1,17}$= 0.226, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,17}$ = 0.526, n.s.).

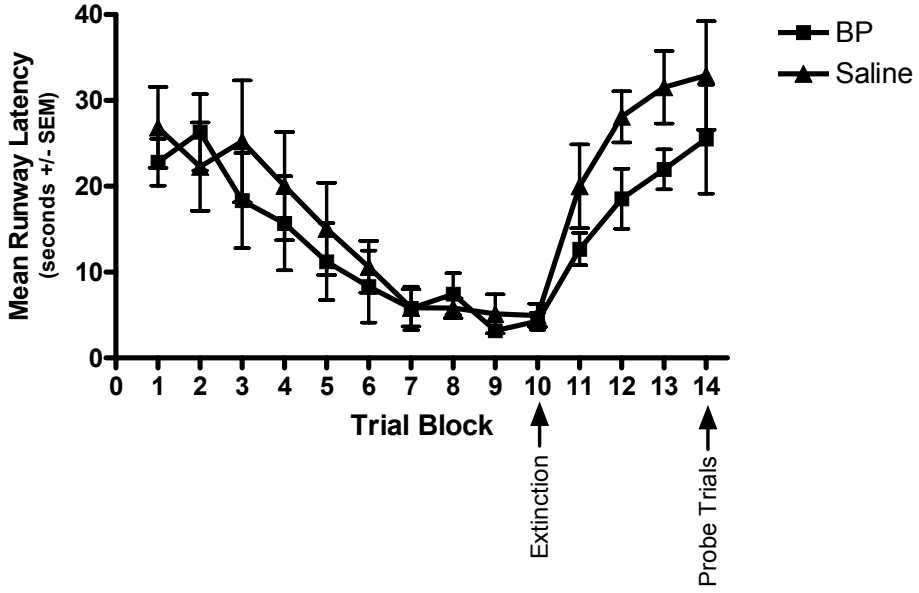

Figure 1.3 The effect of dorsolateral caudate inactivation on response extinction (experiment 1). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. Dorsolateral caudate inactivation significantly attenuated response extinction.

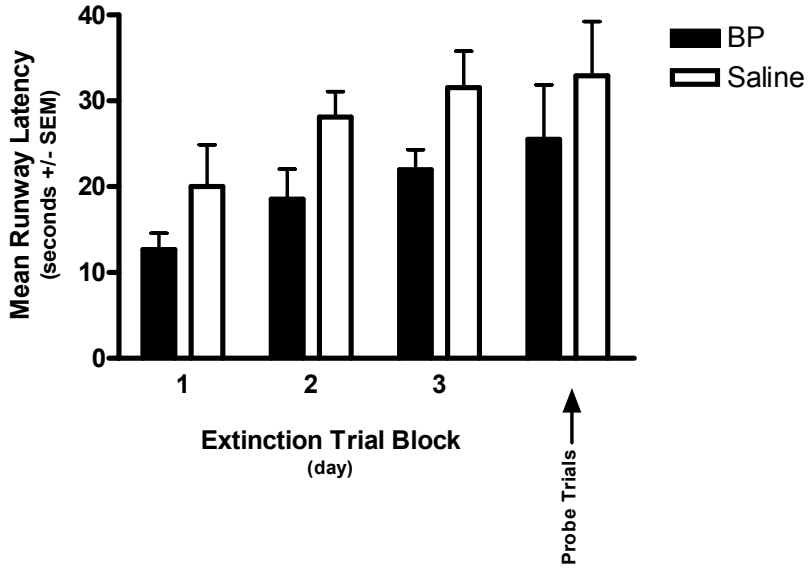

Figure 1.4 Response extinction following dorsolateral caudate inactivation (experiment 1). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. Dorsolateral caudate inactivation significantly attenuated response extinction.

Discussion

The present results demonstrate that following reversible inactivation with bupivacaine of the dorsolateral caudate immediately pre-extinction training in the straight alley maze, response extinction was attenuated while latent extinction remained intact. This selective impairment on response extinction due to caudate inactivation suggests that bupivacaine does not impair general maze behavior, since similar effects would be apparent in latent extinction. Overall, the selective impairment of response extinction can be attributed to a caudate mediated impairment in response extinction. These results are consistent with previous findings indicating a role for the dorsal striatum in the acquisition of stimulus-response habits (Graybiel, 1998; McDonald & White, 1993; Packard & Knowlton, 2002; Knowlton et al., 1996; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002). Further, the involvement of the dorsolateral

caudate suggests that kinesthetic/vestibular cues produced by maze running may contribute to the extinction of the habitual running response. The lack of involvement of the dorsolateral caudate in the acquisition of latent extinction is consistent with findings that demonstrate that the functional integrity of the caudate is not required for cognitive or relational learning (McDonald & White, 1993; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002).

The acquisition of learned behaviors involves multiple memory systems (McDonald & White, 1993; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002) and the present findings indicate that the learning that underlies extinction can also invoke multiple memory systems. Taken together with previous findings from our lab indicating the selective impairment of latent extinction following hippocampal inactivation (Gabriele & Packard, 2006; see figures 1.5, 1.6), the current results demonstrate that the same overt behavior can be extinguished with or without the previously reinforced response and that the learning that underlies latent and response extinction can be neuroanatomically dissociated. Specifically, the hippocampus mediates cognitive latent extinction and the caudate mediates habitual response extinction, effectively demonstrating a double dissociation of two different forms of extinction of the same overt behavior. These results are similar to dissociations between hippocampal and caudate memory systems seen in the acquisition of spatial and habit versions of both the radial arm maze task (Packard, Hirsch, and White, 1989) and the Morris water maze task (Packard & McGaugh, 1996) however this is the first of such dissociations to be demonstrated in extinction behavior.

It is important to note that our previous study investigating the role of the hippocampus in runway extinction demonstrated that hippocampal inactivation produced an effective blockade of latent extinction (Gabriele & Packard, 2006). The current findings indicated that caudate inactivation attenuated response extinction, but a significant extinction effect was still observed. Given that acquisition and extinction were conducted in an open maze with access to extra-maze cues, the hippocampal memory system was still functioning during response extinction and partially able to extinguish responding, however the current impairment indicates that an intact caudate is critical for normal extinction of habitual responding.

Overall, similar to initial acquisition, the learning that underlies extinction involves multiple memory systems. These results provide evidence that utilizing a multiple memory systems approach may be beneficial to understanding the neural bases of extinction behavior.

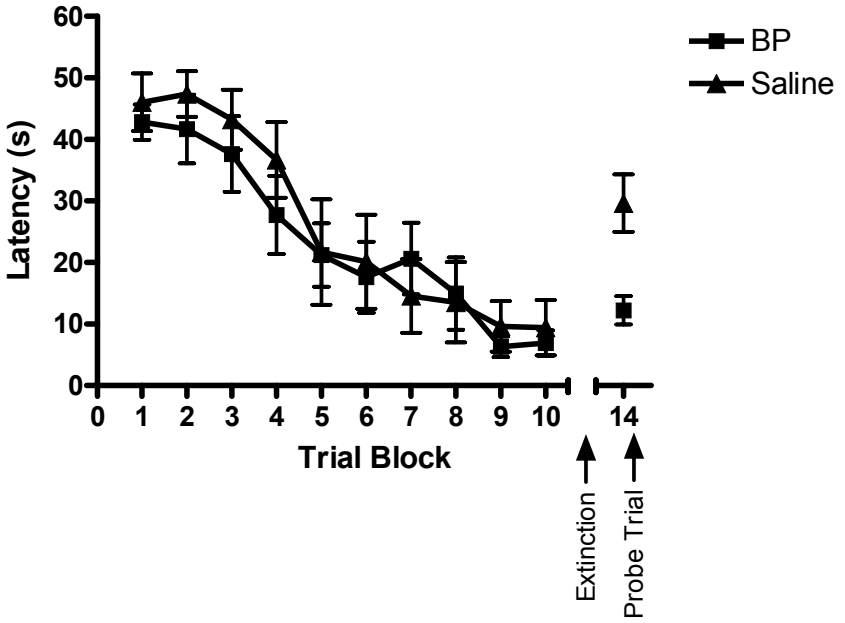

Figure 1.5 The effect of dorsal hippocampus inactivation on latent extinction. Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. BP = 0.75% bupivacaine. *Adapted from Gabriele & Packard, 2006*

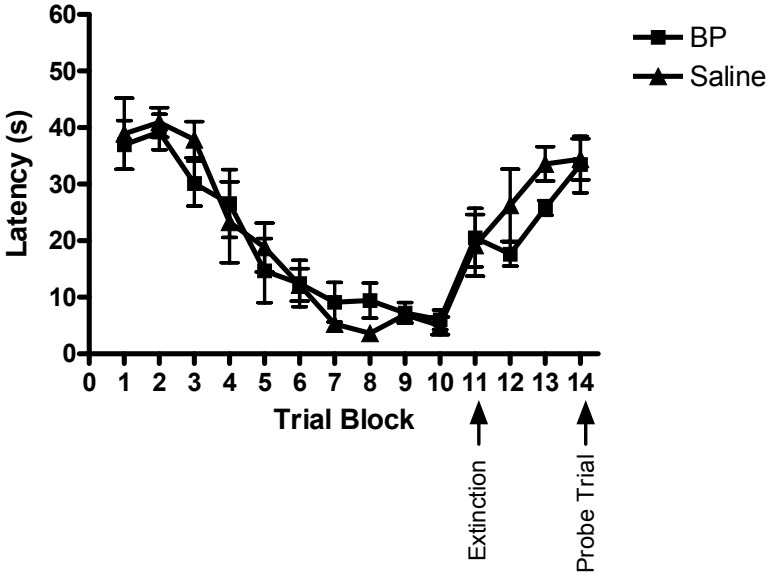

Figure 1.6 The effect of dorsal hippocampus inactivation on response extinction. Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group. *Adapted from Gabriele & Packard, 2006*

CHAPTER IV

EXPERIMENT 2

Introduction

The medial prefrontal cortex (mPFC) is a brain structure that has been implicated in fear extinction recall (Morgan et al., 2003; Hugues et al., 2004; Santini et al., 2004; Sierra-Mercado et al., 2006) through regulation of amygdala-mediated fear conditioning (for review see Sotres-Bayon et al., 2004). Permanent lesions of the ventral medial prefrontal cortex conducted pre-training (Quirk et al., 2000; Lebron et al., 2004; but see also Gerwitz et al., 1997; Farinelli et al., 2006) or reversible inactivation conducted pre-extinction (Sierra-Mercado et al., 2006) do not impair within session extinction however they do cause impairments in retrieval. While ventral mPFC lesions lead to impairments in retrieval, extinction on test day was acquired faster than naïve controls suggesting that extinction was consolidated on the previous day, but was not accessible at test (Quirk et al., 2000). Additionally, recordings from the ventral medial prefrontal cortex during auditory fear conditioning verified a lack of firing during both conditioning and extinction. However on the second day of extinction training, presentation of the tone elicited strong ventral mPFC firing indicating that firing coincided with recall of extinction from the previous day. Additionally, firing was inversely proportional to the level of freezing seen on extinction day 2 demonstrating a direct role for mPFC firing in the reduced freezing as a result of extinction recall (Milad & Quirk, 2002). These findings indicate a clear role for the ventral medial prefrontal cortex in extinction recall, specifically of fear conditioning. These results suggest a potential role for the medial

prefrontal cortex in latent and response extinction behavior in the straight alley.

Additionally, the prefrontal cortex receives inputs from the hippocampus, which may serve to incorporate contextual information (for review see Sotres-Bayon et al., 2004). In the case of fear conditioning extinction, the prefrontal cortex may incorporate contextual information from the hippocampus to order to gate recall of either a fear response or an extinction response of fear inhibition if in the extinction context (Quirk & Mueller, 2008). Given these projections, it is possible that the prefrontal cortex also plays a role in hippocampal-dependent latent extinction (Gabriele & Packard, 2006).

The prefrontal cortex also plays a role in inhibition of previously acquired responses (Ridderinkhof et al., 2004; Broersen & Uylings, 1999; Muir et al., 1996; Kolb, 1984) and therefore may contribute to the inhibition of the runway response acquired during response extinction. Excitotoxic lesions of the medial prefrontal cortex impair performance by causing perseverative responding in a 5 choice serial reaction time task in which animals are trained to nosepoke various lighted locations for food reward (Muir et al., 1996). Similarly, aspiration lesions of the medial prefrontal cortex impaired both spatial reversal learning in which animals are trained to alternate between two runways between sessions for food reward and delayed response performance in which animals are trained to respond after a delay to a light cue indicating which of two food wells contains reward (Kolb et al., 1974). These impairments indicated perserverative responding to the previously reinforced location.

If response inhibition is impaired as a result of compromised medial prefrontal cortical functioning, resulting in response perseveration to a previously reinforced location, then inactivation of the medial prefrontal cortex during response extinction may

result in response perseveration during extinction, if the necessary inhibition cannot occur. Alternately, extinction may be impaired if the animal is unable to recall extinction from the previous day. Experiment 2 aims to examine the potential role of the medial prefrontal cortex in both latent and response extinction.

Method

Subjects

Subjects were 40 adult male Long-Evans rats (275-300 g). All animals received water *ad libitum*.

Apparatus

The straight alley maze described in the general methods is used for experiment 2.

Surgery

Animals received bilateral cannulation surgeries in the medial prefrontal cortex as described in the general methods.

Drugs and Infusions

A 0.75% bupivacaine solution (Abbott Laboratories) is used to produce reversible inactivation of the prefrontal cortex. Control animals are infused with physiological saline. Bilateral intra-PFC infusions are administered as described in the general methods. This infusion procedure was identical to that of our previous study indicating a role for the dorsal hippocampus in latent extinction (Gabriele & Packard, 2006).

Straight-Alley Maze Acquisition and Extinction Training

All acquisition, extinction, and extinction testing procedures were identical to those described in experiment 1. Rats were matched based on latencies to reach the food cup during the last three days of food-rewarded training to form a total of four extinction

groups; two "response" extinction groups (bupivacaine, n = 10; and saline, n = 10), and two "latent" extinction groups (bupivacaine, n = 10; and saline, n = 10).

Results

Latent Extinction

A one-way ANOVA indicated no difference in latent extinction across probe trials for those animals receiving intra-medial prefrontal cortex infusions of bupivacaine (M = 15.475, SEM = 10.125) as compared to controls (M = 15.475, SEM = 9.640) across probe trials ($F_{1,18} = 0.000$, n.s.) (Figures 2.1, 2.2). Further analyses revealed that there was a significant difference between the last day of acquisition training and the probe trails for both animals that received bupivacaine (last acquisition M = 4.116, SEM = 2.074; probe M = 15.475, SEM = 10.125) ($F_{1,18} = 12.079$, $p < 0.005$) and controls (last acquisition M = 6.433, SEM = 3.347; probe M = 15.475, SEM = 9.640) ($F_{1,18} = 7.851$, $p < 0.05$) indicating that both groups displayed a significant extinction effect. Additionally, in a two-way one-repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,18} = 30.646$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for drug treatment ($F_{1,18} = 0.554$, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,18} = 0.157$, n.s.) indicating that both groups acquired the task at a similar rate.

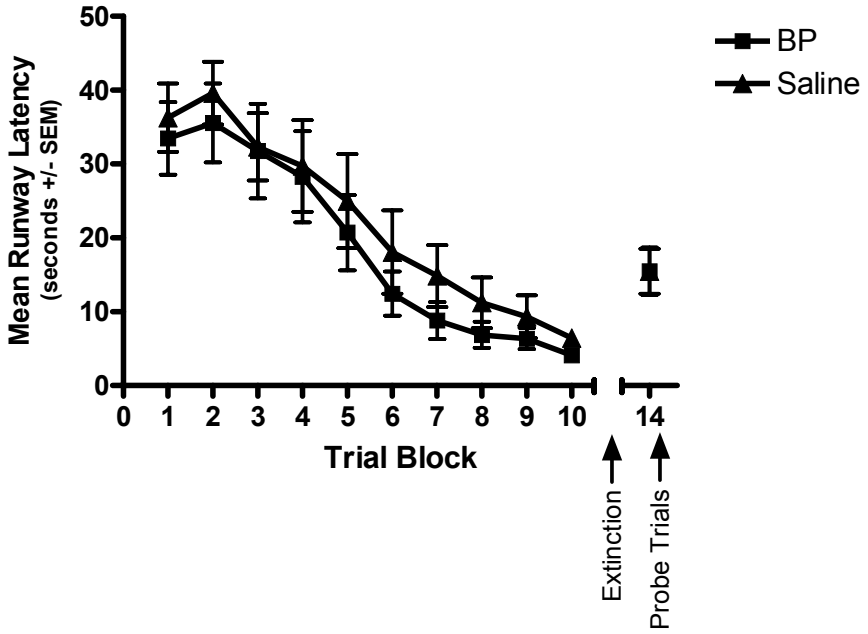

Figure 2.1 The effect of medial prefrontal cortex inactivation on latent extinction (experiment 2). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Medial prefrontal cortex inactivation did not affect latent extinction. BP = 0.75% bupivacaine

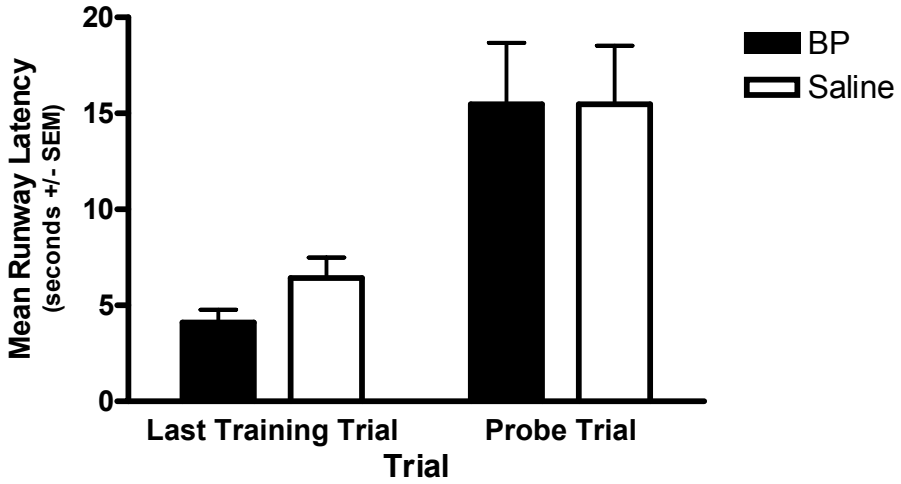

Figure 2.2 Latent extinction following medial prefrontal cortex inactivation (experiment 2). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Medial prefrontal cortex inactivation did not affect latent extinction.

Response Extinction

A one-way ANOVA indicated no difference in response extinction across probe trials for those animals receiving intra-medial prefrontal cortex infusions of bupivacaine (M = 28.400, SEM = 11.911) as compared to controls (M = 31.576, SEM = 13.413) ($F_{1,18} = 0.313$, n.s.) (Figures 2.3, 2.4). Additionally, a two-way one-repeated measures ANOVA found no differences in groups based on drug treatment for acquisition of extinction, there was a significant main effect for extinction day ($F_{2, 18} = 4.310$, $p < 0.05$), but no significant effect for drug treatment ($F_{1, 18} = 1.041$, n.s.) or interaction between extinction day and drug treatment ($F_{2,18} = 0.433$, n.s.) were found, indicating that both groups extinguished at a similar rate. Additionally, there was a significant difference between the last day of acquisition training and the probe trails for both animals that received bupivacaine (last acquisition M = 6.167, SEM = 5.150; probe M = 28.400, SEM = 11.911) ($F_{1,18} = 28.175$, $p < 0.001$) and controls (last acquisition M = 7.117, SEM = 6.363; probe M = 31.576, SEM = 13.413) ($F_{1,19} = 27.142$, $p < 0.001$) indicating that both groups displayed a significant extinction effect. Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9, 18} = 47.777$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1,18} = 0.001$, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,18} = 0.357$, n.s.).

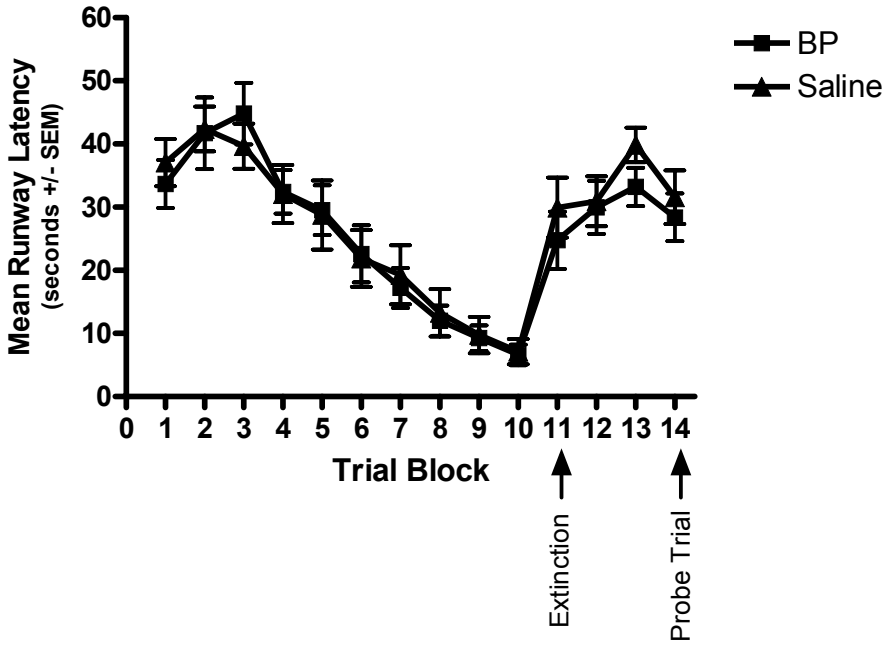

Figure 2.3 The effect of medial prefrontal cortex inactivation on response extinction (experiment 2). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Medial prefrontal cortex inactivation did not affect response extinction.

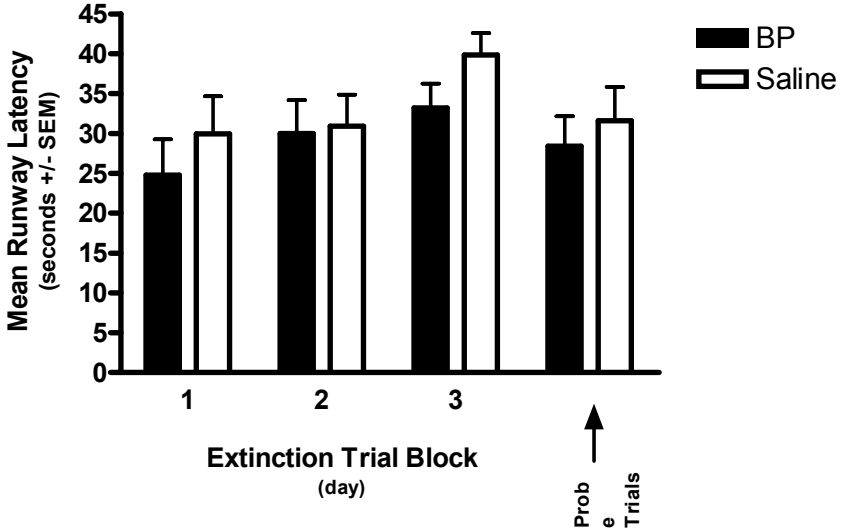

Figure 2.4 Response extinction following medial prefrontal cortex inactivation (experiment 2). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Medial prefrontal cortex inactivation did not affect response extinction.

Discussion

The results of the present study indicate that, under the current training parameters, reversible neural inactivation of the medial prefrontal cortex immediately pre-extinction training does not affect acquisition of either latent or response extinction in the straight alley task. These findings indicate that the medial prefrontal cortex may not be involved in generalized extinction or extinction recall of all behaviors further supporting the multiple memory systems theory of extinction that, similar to initial acquisition, different brain systems mediate different types of extinction behavior.

Medial prefrontal cortex inactivation did not affect extinction recall for either type of extinction. This lack of effect was demonstrated by no apparent differences between groups in the second and third extinction days of response extinction and at the probe trial. Additionally, due to the nature of the latent extinction paradigm, there are no within session extinction measures, only extinction recall on the probe trials, is appears that the medial prefrontal cortex is not involved in recall of latent extinction. However, since the medial prefrontal cortex was inactivated only during extinction training and not at the probe trials, it is possible that inactivating the mPFC immediately prior to the probe trials would lead to impairments in responding. However, this result is unlikely given the lack of effect of mPFC inactivation over recall of response extinction. Additionally, previous studies examining fear extinction recall demonstrated impairments following lesions conducted both pre-acquisition and pre-extinction (Quirk et al., 2000; Lebron et al., 2004; Sierra-Mercado et al., 2006). It is possible, given the high connectivity with the amygdala (for review see Sotres-Bayon et al., 2004) that the medial prefrontal cortex selectively affects extinction recall of amygdala mediated learning (Falls et al., 2002; Walker et al.,

2002; Fuchs et al., 2002; Schroeder & Packard, 2004; Boutreau et al., 2006) such as fear conditioning (Quirk et al., 2000; Lebron et al., 2004; Sierra-Mercado et al., 2006) and conditioned place preference (Hsu & Packard, 2008).

Although latent extinction was not affected, medial prefrontal cortex inactivation has impaired learning on several hippocampus dependent tasks involving spatial learning. However, it appears that the medial prefrontal cortex may be selectively involved in spatial working memory (Taylor et al., 2003; Touzani et al., 2007; Yoon et al., 2008), and perhaps specifically working memory for spatial response information (Kesner et al., 1996). Studies examining spatial memory in the Morris water maze task (Sloan et al., 2006; LaCroix et al., 2002; de Bruin et al., 1994) and the radial arm maze (Yoon et al., 2008) have demonstrated that medial prefrontal cortex inactivation selectively impair working memory and not spatial reference memory indicating that the mPFC is not involved in the storage of spatial information that remains constant throughout training (LaCroix et al., 2002). Given that acquisition of latent extinction relies on spatial reference memory rather that spatial working memory, the lack of effect following medial prefrontal cortex inactivation is not surprising. The medial prefrontal cortex also does not appear to be required in the processing of spatial information (de Bruin et al., 1994) indicating that the association between the spatial cues of the goalbox and non-reward that occurs during latent extinction should not be affected. However, impairments following medial prefrontal cortex inactivation are seen in spatial tasks that require behavioral flexibility such as spatial reversals (LaCroix et al., 2002; de Bruin, 1994; Kolb, 1974) Since latent extinction relies on neither spatial working memory or within

session behavioral flexibility, it follows that the medial prefrontal cortex should not be required for latent extinction to occur.

However, given the role of the medial prefrontal cortex in response inhibition, the lack of involvement of the mPFC in response extinction is a little more surprising. While medial prefrontal cortex inactivation leads to response perseveration in several tasks (Ridderinkhof et al., 2004; Broersen & Uylings, 1999l; Muir et al., 1996; Kolb, 1974), some have argued that this perseverative responding is the result of impaired behavioral flexibility based on spatial information (Kolb et al., 1974). Since response extinction is a habitual form of extinction that does not require spatial information this could indicate a lack of involvement of the medial prefrontal cortex. However, while the medial prefrontal cortex is not required for response extinction under the current training parameters, this does not eliminate the potential for the involvement of the medial PFC during runway extinction. Further studies are necessary to elucidate the potential role of the prefrontal cortex in response extinction of runway behavior.

CHAPTER V

EXPERIMENT 3

Introduction

The basolateral amygdala (BLA) has been implicated in mammalian memory processes including extinction of learned behaviors (Packard & Teather, 1998; Packard & Cahill, 2001; Falls et al., 1992; Walker et al., 2002; Schroeder & Packard, 2003; 2004; in both the extinction of fear conditioning). Additionally, given the double dissociation of hippocampal dependent latent extinction and caudate dependent response extinction as seen from previous studies (Gabriele & Packard, 2006) and Experiment 1, it is important to consider the modulatory role the amygdala plays over multiple memory systems (for review see Packard & Cahill, 2001). The basolateral amygdala has projections to both the dorsal hippocampus (Pitkanen et al., 2000) and the striatum (Kita & Kitai, 1990). Additionally, the basolateral amygdala plays a role in hippocampal LTP. Reversible and irreversible lesions of the basolateral amygdala attenuated LTP in the dentate gyrus of the hippocampus while high frequency stimulation of the basolateral amygdala facilitated the induction of LTP in the dentate gyrus, indicating that LTP in the hippocampus is modulated by the amygdala (Abe, 2001). Further behavioral evidence has also indicated that the amygdala plays a modulatory role over the hippocampus and caudate memory systems. While post-training intra-hippocampal administration of amphetamine enhances retention in the spatial water maze task and post-training intra-caudate administration of amphetamine enhances retention in the cued water maze task, amphetamine administration in the amygdala enhances retention in both tasks indicating a modulatory effect over both systems (Packard, Cahill, & McGaugh, 1994). Additionally, while

concurrent administration of lidocaine into either the hippocampus or the caudate block this enhancement effect, pre-retention test administration of lidocaine into the amygdala does not indicating that the memory enhancing effect is due to efferent projections from the amygdala and that memory for these tasks is not stored in the amygdala (Packard & Teather, 1998). Since the amygdala plays a modulatory role over multiple memory systems during acquisition, it is possible that this modulation extends to extinction.

It has previously been established that the amygdala plays a role in the extinction of stimulus-affect learning, specifically extinction of fear conditioning and conditioned place preference behavior (Falls et al., 2002; Walker et al., 2002; Fuchs et al., 2002; Schroeder & Packard, 2004; Boutreau et al., 2006). Since lesions of the basolateral amygdala block both the acquisition and expression of conditioned fear (for review see LeDoux, 2003; Maren, 2001), lesion studies were not effective to examine extinction given that it would be difficult to determine whether a reduction on freezing behavior is due to an extinction of conditioned fear or a blockade in the expression of conditioned fear. However, several studies examining the glutamatergic neurotransmitter system have indicated a role for the amygdala in extinction of conditioned fear (Falls et al., 2002; Walker et al., 2002). Intra-amygdala administration of NMDA receptor antagonists impair extinction of fear potentiated startle (Falls et al., 1992) while agonists enhance it (Walker et al., 2002). Given the role of NMDA receptors in LTP, it is possible that LTP-like processes in the amygdala may underlie the acquisition of extinction behavior, specifically extinction of fear-potentiated startle. Additional evidence that the amygdala is involved in the extinction of stimulus-affect association is seen in studies examining conditioned place preference. Excitotoxic lesions of the basolateral amygdala impaired

extinction of a cocaine conditioned place preference (Fuchs et al., 2002) while intra-amygdala muscarinic receptor agonism enhanced extinction of an amphetamine CPP (Schroeder & Packard, 2004) and intra-amygdala NMDA receptor agonism enhanced extinction of a cocaine CPP (Boutreau et al., 2006). These findings demonstrate that the amygdala is involved in extinction of stimulus-affect associations and these effects are mediated by intra-amygdala NMDA and acetylcholine receptors. However, electrolytic amygdala lesions have also impaired both acquisition and extinction of a runway task indicating that the role of the amygdala may extend to extinction of appetitively motivated tasks (Kemble & Beckman, 1970).

Further evidence that may indicate the potential involvement of the basolateral amygdala in latent or response extinction is the role of the BLA in both sensitivity to reward change and frustrative non-reward (Henke, 1977; Salinas, Packard & McGaugh, 1993; Salinas & McGaugh, 1998). Reversible inactivation of the amygdala attenuated sensitivity to reward reduction in a straight alley maze as compared to controls (Salinas, Packard, & McGaugh, 1993). Additionally, while permanent pre-training electrolytic lesions of the amygdala do not prevent increased latencies in response to reward reduction on the initial shift day, this response was eliminated on subsequent test days, indicating that the amygdala is necessary for conditioning the aversive or frustrating properties of reward reduction (Salinas & McGaugh, 1998). Similarly, the amygdala plays a role in frustrative non-reward. The effect of frustrative non-reward is demonstrated when, in a double runway maze where a goalbox separates the first and second runways, unexpected non-reward in the first goal box produce accelerated latencies to the second goalbox. Amygdala lesions have been shown to eliminate this

effect (Henke & Maxwell, 1973; Henke, 1977). Therefore, it is possible that the amygdala could contribute to the association of any frustrative or aversive properties that either response extinction or confinement in the goalbox without reward might produce.

While previous research has indicated the potential involvement of the amygdala in response extinction (Kemble & Beckman, 1970) the role of the basolateral amygdala in extinction that occurs without the explicit performance of the previously reinforced response is unknown. Therefore experiment 3 aims to examine whether latent and/or response extinction is dependent on the basolateral amygdala.

Method

Subjects

Subjects were 36 adult male Long-Evans rats (275-300 g). All animals received water *ad libitum*.

Apparatus

The straight alley maze described in the general methods is used for experiment 3.

Surgery

Animals received bilateral cannulation surgeries in the basolateral amygdala as described in the general methods.

Drugs and Infusions

A 0.75% bupivacaine solution (Abbott Laboratories) was used to produce reversible inactivation of the amygdala. Control animals were infused with physiological saline. Bilateral intra-amygdala infusions were administered as described in the general methods. This infusion procedure was identical to that of our previous study indicating a role for the dorsal hippocampus in latent extinction (Gabriele & Packard, 2006).

Straight-Alley Maze Acquisition and Extinction Training

All acquisition, extinction, and extinction testing procedures were identical to those described in experiment 1. Rats were matched based on latencies to reach the food cup during the last three days of food-rewarded training to form a total of four extinction groups; two "response" extinction groups (bupivacaine, n = 8; and saline, n = 7), and two "latent" extinction groups (bupivacaine, n = 11; and saline, n = 10).

Results

Latent Extinction

A one-way ANOVA indicated no difference in latent extinction across probe trials for those animals receiving intra-basolateral amygdala infusions of bupivacaine (M = 20.272, SEM = 7.963) as compared to controls (M = 17.375, SEM = 8.877) across probe trials ($F_{1,19} = 0.440$, n.s.) (Figures 3.1, 3.2). Further analyses revealed that there was a significant difference between the last day of acquisition training and the probe trails for both animals that received bupivacaine (last acquisition M = 7.001, SEM = 5.362; probe M = 20.272 SEM = 7.963) ($F_{1,20} = 21.022$, $p < 0.001$) and controls (last acquisition M = 6.30, SEM = 5.460; probe M = 17.375, SEM = 8.877) ($F_{1,18} = 11.294$, $p < 0.005$) indicating that both groups displayed a significant extinction effect. Additionally, in a two-way one-repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,19} = 31.357$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for drug treatment ($F_{1,19} = 0.151$, n.s.) indicating no significant differences in latencies between treatment groups.

Also, a significant interaction effect was not observed between day and treatment ($F_{9,19}$ = 0.423, n.s.) indicating that both groups acquired the task at a similar rate.

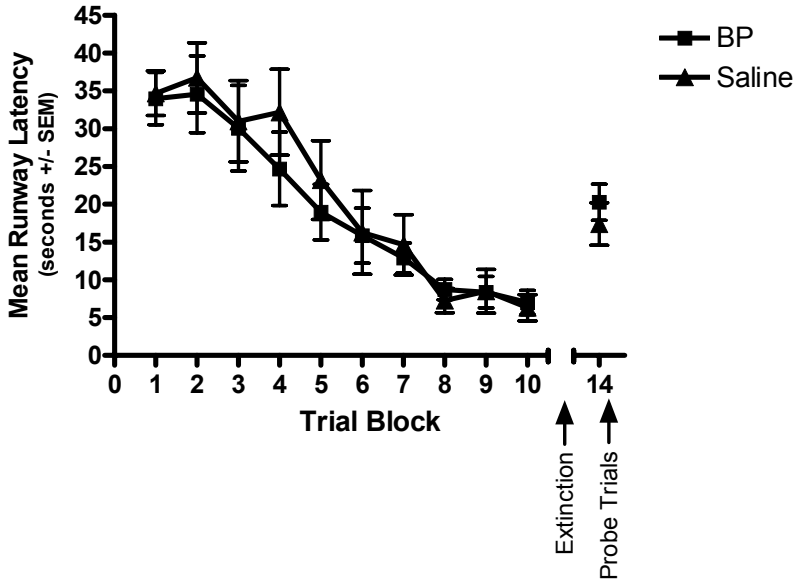

Figure 3.1 The effect of basolateral amygdala inactivation on latent extinction (experiment 3). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Basolateral amygdala inactivation did not affect latent extinction. BP = 0.75% bupivacaine

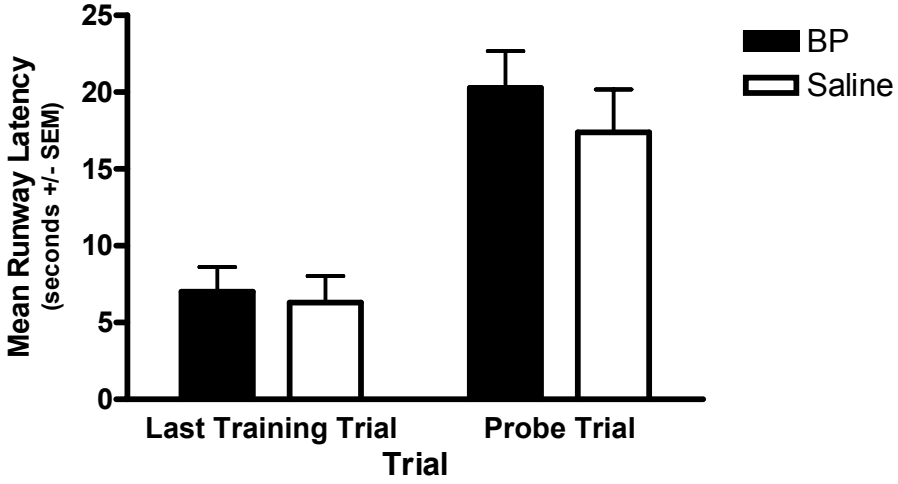

Figure 3.2 Latent extinction following basolateral amygdala inactivation (experiment 3). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Basolateral amygdala inactivation did not affect latent extinction.

Response Extinction

A one-way ANOVA indicated no difference in response extinction across probe trials for those animals receiving intra-basolateral amygdala infusions of bupivacaine (M = 34.25, SEM = 14.332) as compared to controls (M = 29.833, SEM = 10.670) ($F_{1,13}$ = 0.446, n.s.) (Figures 3.3, 3.4). Additionally, a two-way one-repeated measures ANOVA found no differences in groups based on drug treatment for acquisition of extinction, there was a significant main effect for extinction day ($F_{2, 13}$ = 0.011, $p < 0.05$), but no significant effect for drug treatment ($F_{1, 13}$ = 0.042, n.s.) or interaction between extinction day and drug treatment ($F_{2,13}$ = 0.492, n.s.) were found, indicating that both groups extinguished at a similar rate. Additionally, there was a significant difference between the last day of acquisition training and the probe trails for both animals that received bupivacaine (last acquisition M = 7.124, SEM = 3.830; probe M = 34.250, SEM = 14.332) ($F_{1,14}$= 26.746, $p < 0.001$) and controls (last acquisition M = 7.120, SEM = 9.332; probe M = 29.832, SEM = 10.670) ($F_{1,12}$ = 17.970 p <0.005) indicating that both groups displayed a significant extinction effect. Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9, 13}$ = 29.066, $p <$ 0.001) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1,13}$= 0.024, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,13}$ = 0.503, n.s.).

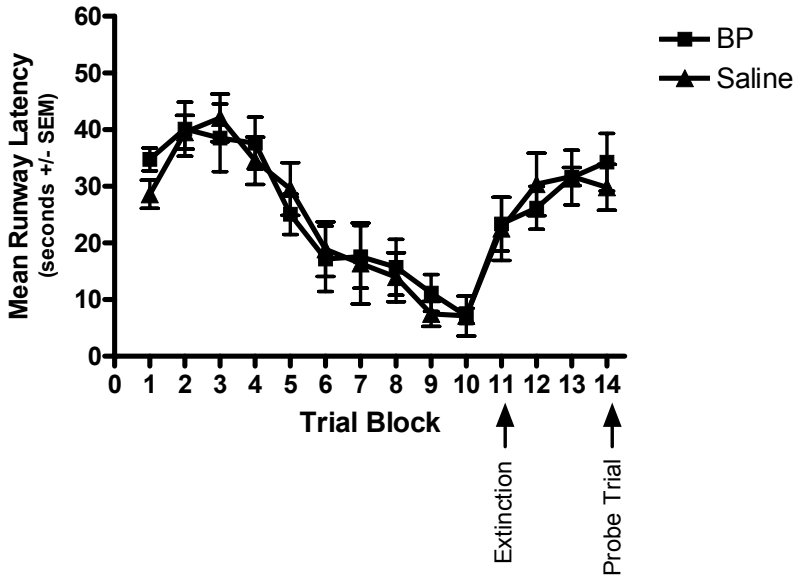

Figure 3.3 The effect of basolateral amygdala inactivation on response extinction (experiment 3). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Basolateral amygdala inactivation did not affect response extinction.

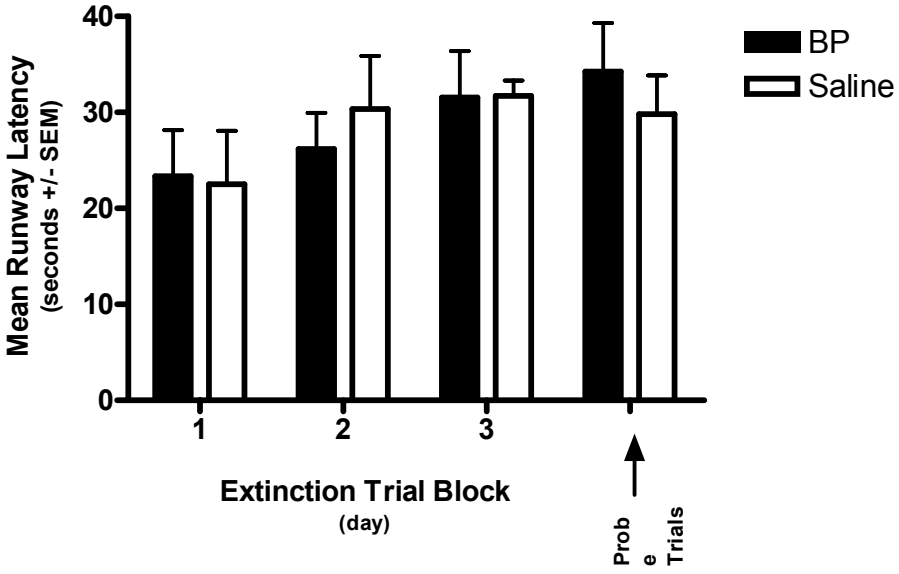

Figure 3.4 Response extinction following basolateral amygdala inactivation (experiment 3). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Basolateral amygdala inactivation did not affect response extinction.

Discussion

The findings of the present experiment indicate that, under the current training parameters, reversible neural inactivation of the basolateral amygdala immediately pre-extinction training does not affect either latent or response extinction in the straight alley maze. These findings are consistent with those that indicate that while the amygdala plays a modulatory role over spatial and stimulus-response habit learning, it is not necessary to acquire these types of learning, nor is it the site of storage (Packard et al., 1996; Packard, Cahill, & McGaugh, 1996; Packard & Teather, 1998; McDonald & White, 1993; White & McDonald, 2002). These findings also indicate that the basolateral amygdala is not involved in a generalized extinction of all behaviors, further supporting the multiple memory systems theory of extinction that, similar to initial acquisition, different brain systems mediate different types of extinction behavior.

While the current training parameters do not necessitate the use of the amygdala during acquisition of extinction behavior in a straight alley maze, it is possible that there may be some situation in which the amygdala is involved. Since a single latent extinction leads to an accelerated responding attributed to a frustration effect (Jones, 1970), it is possible that the amygdala underlies this behavior. Additionally, since the amygdala is implicated in emotionally mediated learning (Gallagher & Chiba, 1996), increasing the emotional salience of the extinction event could recruit the amygdala. For example, an increased hunger drive was found to enhance latent extinction as compared to latent extinction conducted under lower or no hunger drive (Moltz & Maddi, 1956). The effect could be the result of an amygdala modulated enhancement of latent extinction under

more emotionally salient conditions. It is also possible that this effect potentially attributable to the amygdala could also be observed in response extinction.

Similarly, given to the modulatory role of the amygdala over the hippocampal and caudate memory systems that is required for both drug enhancement and drug impairment (Packard & Teather, 1998; Packard et al., 1996; Packard, Cahill, & McGaugh, 1996; , memory enhancing drugs such as amphetamine administered intra-amygdala could have the effect similar that seen in the acquisition of hippocampal-dependent and caudate-dependent learning of enhancing both latent and response extinction (Packard & Teather, 1998). Further, the amygdala affects the relative use of multiple memory systems through the effects of stress hormones such as norepinephrine (Cahill & McGaugh, 1991) and glucocorticoids, through a stress produced impairment of hippocampal dependent learning (Kim et al., 2001) and a facilitate switch to habitual response learning (Packard & Wingard, 2004). Therefore, it would be predicted that inducement of acute stress through intra-amygdala anxiogenic drug administration for example, would cause impairments in latent extinction.

Overall, while the functional integrity of the basolateral amygdala is not necessary for the acquisition of either latent or response extinction under the present training parameters, its potential modulatory role over these two types of extinction has yet to be fully examined. For example, although the amygdala is not required for latent or response extinction to occur, it could still contribute to the potentially aversive or frustrative effects of non-reward during extinction and under situations in which inducing a stronger affective state would have the potential for recruiting amygdalar function.

CHAPTER VI

EXPERIMENT 4

Introduction

Although our previous work demonstrates that the functional integrity of the hippocampus is required for latent extinction through reversible inactivation with bupivacaine (Gabriele & Packard, 2006), these findings do not provide any information concerning the neurochemical basis of this form of cognitive learning. Several neurotransmitter systems have been implicated in extinction learning (for review see Mason 1983) and recent studies have indicated a role similar to initial learning for glutamatergic neurotransmission (for review see Davis and Myers 2002). For example, administration of D-cycloserine (DCS), a partial agonist at the strychnine insensitive glycine binding site of the NMDA receptor enhances extinction when administered systemically either pre- or post-extinction for both conditioned fear (Walker et al. 2002; Ledgerwood et al. 2003; 2005; Woods & Bouton, 2006; for review see Richardson, Ledgerwood & Cranney, 2004) and cocaine conditioned place preference (Botreau et al., 2006) indicating a role in the extinction of amygdala-mediated stimulus-affect learning. Importantly, in addition to enhancing extinction of fear, D-cycloserine administration also reduces fear for non-extinguished stimuli, demonstrating important clinical implications for the use of DCS for generalization of extinction (Ledgerwood et al., 2005). Taken together with recent clinical findings that have successfully employed D-cycloserine to facilitate extinction of fear responses in human anxiety disorders such as phobias and social anxiety disorder (Ressler et al. 2004; Hofmann et al. 2006), DCS has

demonstrated significant potential as a pharmacotherapeutic approach toward the extinction of maladaptive behaviors.

While some extinction therapies used in clinical settings involve training in the presence of overt behavioral responses, other approaches are based on developing *cognitive* control over maladaptive behaviors, and this form of learning may not require overt responding during extinction training. Given that latent extinction is selectively involved in cognitive learning and memory (Seward and Levy 1949; Gabriele and Packard 2006), elucidation of the neurochemical bases of this form of extinction learning may have potential implications for therapeutic strategies used in the treatment of various psychopathologies. Further, D-cycloserine administration enhances initial acquisition of hippocampal dependent tasks including trace eyeblink conditioning and Morris water maze (Thompson et al., 1992; Lelong et al., 2001) indicating a potential for enhancement of hippocampus dependent extinction learning. Experiment 4 examined whether post-extinction training injections of D-cycloserine are effective in enhancing memory consolidation underlying hippocampus-dependent latent extinction.

Method

Subjects

Subjects were 26 adult male Long-Evans rats (275-300 g). All animals received water *ad libitum*.

Apparatus

The straight alley maze described in the general methods is used for experiment 4.

Drugs and Infusions

D-cycloserine (Sigma Pharmaceuticals) was dissolved in physiological saline and injected intraperitoneally at a dose of 15 mg/kg. This dose was selected based on previous studies examining the effectiveness of DCS on extinction behavior. 15 mg/kg was the dose most commonly found to be effective in these studies (Botreau et al. 2006; Lee et al. 2006; Parnas et al. 2005; Walker et al. 2002; Ledgerwood et al. 2003). Control animals were injected with physiological saline.

Straight-Alley Maze Acquisition Training

Straight alley maze acquisition training was identical to the methods described in experiment 1.

Extinction Training: General Procedure

Twenty-four hours following the completion of acquisition training (i.e. day 11), rats were matched based on latencies to reach the food cup during the last three days of food-rewarded training to form two extinction groups; animals receiving DCS 15 mg/kg (n = 9) or saline (n = 12). During latent extinction training, rats were placed by the experimenter facing the empty food cup in the goal end of the maze and were confined for 60 seconds by placement of a clear Plexiglas shield (8 inches from the end of the maze arm). Following confinement, rats were removed from the maze and placed in an opaque holding box located on a table adjacent to the maze for a 30 second inter-trial interval. Latent extinction training was administered in a *single session* (6 trials) in order to better allow for the potential enhancement effect, and rats received injections of saline or DCS immediately post extinction training.

Twenty-four hours following the completion of extinction training (i.e. day 12), all rats were given an additional four extinction "probe" trials in which they were placed in the start end of the maze and latency to reach the empty food cup is recorded. These four trials allowed for an assessment of the effectiveness of the latent extinction procedure in saline and DCS treated rats. An additional group of animals (n = 5) received DCS injections (15 mg/kg) two hours post extinction training as a control group to control for any potential pro-active non-mnemonic effects (e.g. sensory, motor, or motivational) of DCS on extinction, and to determine whether DCS influences memory consolidation in a time-dependent manner.

Results

A two-way one repeated measures ANOVA (Group X Trial) comparing the latencies to reach the food cup during acquisition revealed a non-significant interaction ($F_{9, 23} = 0.375$, n.s.) and a non-significant main effect of Group ($F_{2, 23} = 0.030$, n.s). A significant Trial effect ($F_{9, 23} = 43.192$, $p < 0.001$) revealed that the latency to reach the food cup during acquisition improved in both groups at a similar rate, and therefore any drug-induced differences in extinction cannot be due to differential rates of acquisition between control and DCS-treated rats.

In order to examine whether latent extinction training produced significant extinction of runway behavior, a comparison of the mean latency to reach the food cup on the final day of acquisition training and the latencies across the four extinction probe trials was conducted. One-way ANOVA'S comparing runway latencies revealed that animals receiving saline ($F_{1, 23} = 14.537$, $p < 0.01$), or DCS ($F_{1, 17} = 18.741$, $P < 0.01$) showed a significant latent extinction effect. In addition, a one-way ANOVA computed

on the mean latency to reach the food cup across the four extinction probe trials revealed a significant group difference ($F_{1, 20} = 5.037$, $p < .05$), indicating that peripheral administration of DCS enhanced latent extinction relative to saline controls (Figure 4.1).

Peripheral injections of DCS administered two hours post-extinction training did not enhance latent extinction. A one-way ANOVA revealed a significant group difference between the mean runway latency across the four probe trials in rats that received DCS immediately post-extinction training and those that received DCS injections administered two hours post-extinction training ($F_{1, 12} = 5.545$, $p < .05$). Additionally, a one-way ANOVA revealed no significant differences between the mean runway latency across the four probe trials in saline rats and those that received DCS injections administered two hours post-extinction training ($F_{1, 16} = 1.064$, n.s.). This finding suggests that DCS enhanced a time-dependent memory consolidation process underlying latent extinction (McGaugh 1989).

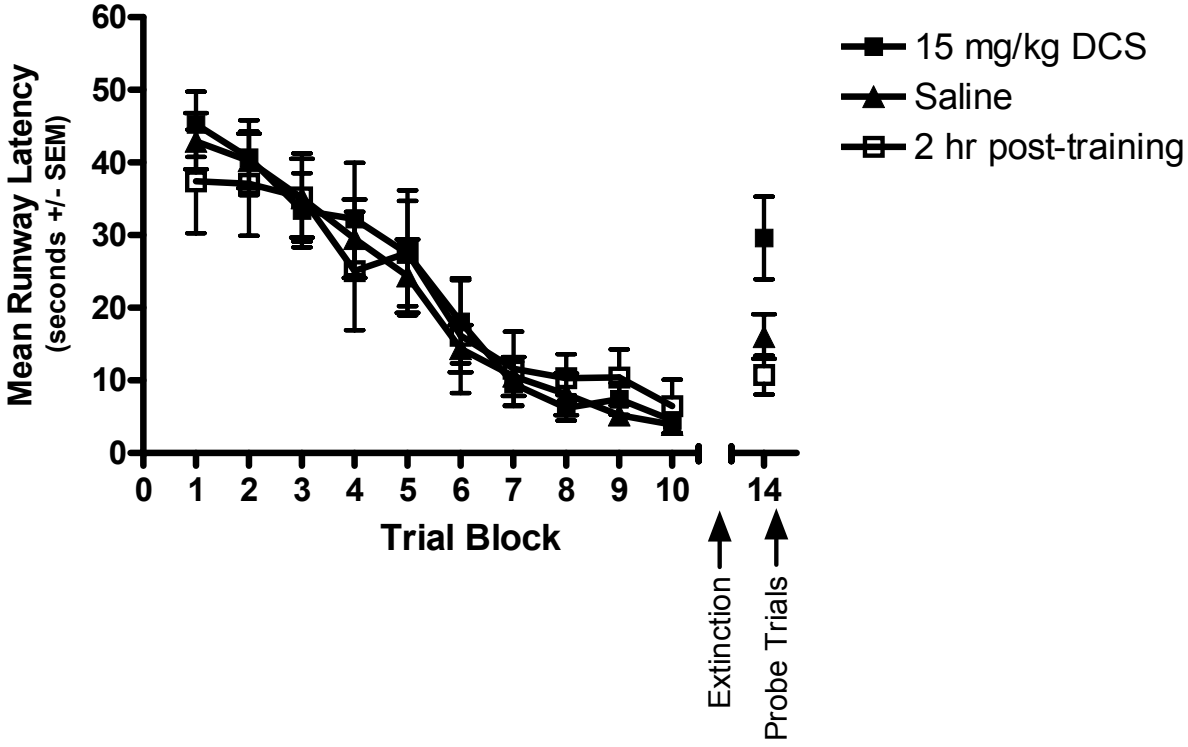

Figure 4.1 The effect of peripheral saline or D-cycloserine on latent extinction. Mean +/- standard error of the mean (SEM) of latency (in seconds) to reach the food cup during the acquisition and extinction probe trials by treatment group (experiment 4). D-cycloserine administered immediately post-extinction training enhanced latent extinction.

Discussion

The present findings indicate that post-training administration of D-cycloserine enhances memory consolidation underlying latent extinction of runway behavior in a straight-alley maze. The results are consistent with evidence that DCS facilitates extinction in other learning tasks including those involving conditioned fear (e.g. Walker et al. 2002; Ledgerwood et al. 2003; 2005; Richardson et al. 2004; Woods & Bouton, 2006) and conditioned place preference behavior (Botreau et al. 2006) and enhances initial acquisition in hippocampus-dependent tasks (Thompson et al., 1992; Lelong et al., 2001). During latent extinction training, the previously reinforced response (e.g. an approach response in a maze runway) is not overtly performed. Therefore, in addition to tasks involving "traditional" *response* extinction training, the present findings extend the learning situations in which DCS can influence extinction behavior to those not requiring the overt response during extinction such as latent extinction.

During memory retrieval, memories may become labile and undergo a reconsolidation process (Nader et. al. 2000). According to the reconsolidation hypothesis, post-extinction training D-cycloserine injections may potentially enhance extinction by *impairing* memory for the original learning, rather than *enhancing* memory for the new learning that can occur during extinction. However, this hypothesis appears inconsistent with findings that post-training DCS administration also enhances memory consolidation for the learning that occurs during initial task acquisition (Hughes 2004; Monahan et. al. 1989). Additionally, following conditioned emotional response training, in which barpress responding for food reward is suppressed during the presentation of a conditioned fearful stimulus, DCS enhances extinction but does not impair renewal,

indicating that the original association is still intact (Woods and Bouton 2006). In the current study, DCS was ineffective when injected two hours post-training, indicating that the drug facilitates memory consolidation in a time dependent manner, and not via an influence on non-mnemonic (e.g. sensory, motor, or motivational) factors (McGaugh 1989).

Glutamatergic NMDA receptors have been implicated in memory consolidation during initial learning in several tasks. For example, post-training administration of DCS enhances memory consolidation during inital task acquisition (Hughes 2004; Monahan et al. 1989). In addition, post-training administration of the NMDA receptor antagonists MK-801 (Packard and Teather 1997a) or AP-5 (Packard and Teather 1997b) impairs hippocampus-dependent spatial memory, and peripheral pre-training injections of DCS facilitate the acquisition of hippocampus-dependent trace eye blink conditioning in rabbits (Thompson et al. 1992). Taken together with our previous findings indicating that latent extinction is mediated by the hippocampus (Gabriele and Packard 2006), it is possible that this brain structure is the site of action for the enhancing effects of peripheral administration of DCS on latent extinction. Further studies are necessary to determine whether direct intra-hippocampal administration of DCS would also enhance consolidation of latent extinction. In sum, the present findings provide further evidence that memory consolidation during initial task acquisition and the new learning that occurs during extinction can similarly involve NMDA receptor function (e.g. Falls et al. 1992) and expands the forms of extinction behavior facilitated by D-cycloserine to those in which no overt response in made during extinction training. It is not yet known how other

neurotransmitter systems may be involved in the acquisition and consolidation of latent extinction learning.

CHAPTER VII

EXPERIMENT 5

Introduction

Latent extinction is unlike traditional extinction paradigms in that it does not require the overt response to be made. However, most latent extinction studies have used dual solution tasks such as the runway or T-maze (Seward & Levy, 1949; Denny & Ratner, 1959; Hughes et al., 1960; Dyal, 1962; Dyal, 1963) which can be acquired using either cognitive or habit memory, therefore the animal could use either learning system for extinction. The question arises then whether requiring the sole use of either spatial or stimulus-response learning during acquisition would influence the relative use of multiple memory systemd during extinction.

In the water maze single-solution place-task, the animal is trained in a T-maze configuration to find a hidden platform in a consistent spatial location from varying start points. Because this task requires the animal to use a spatial information to locate the hidden platform, it follows that extinction could be acquired spatially via latent extinction by confining the animal in the location previously occupied by the hidden platform. However, response extinction would also be possible since it would be acquired in the same manner as the initial task acquisition. Since previous research from our lab has dissociated latent and response extinction following hippocampal inactivation in the straight alley maze task (Gabriele & Packard, 2006), experiment 5a explores the role of the hippocampus in these two forms of extinction following acquisition in the single solution place task and whether a similar dissociation would be evident.

Several studies have shown that glutamatergic NMDA receptors play a significant role in extinction behavior (Szapiro, et al., 2003; Davis & Myers, 2002; Walker & Davis, 2002, for review see Castellano, et al., 2001). More specifically, the partial NMDA agonist D-cycloserine has been found to enhance hippocampal dependent learning (Thompson, et al., 1992; Lelong et al., 2001) including latent extinction in the runway (Gabriele & Packard, 2007, experiment 4) indicating that NMDA receptors play a role in latent extinction. Additionally, intra-hippocampal administration of the competitive NMDA receptor antagonist 2-amino-5-phosphopentanoic acid (AP5) has been used to impair hippocampal dependent learning in several tasks (Packard & Teather, 1997; Steele & Morris, 1999; Liang, et al., 1994; Yoshihara & Ichitani, 2004; Wanisch, et al., 2005). Experiment 5a will also address the role of hippocampal NMDA receptors in both latent and response extinction following acquisition in the single solution place task through reversible inactivation of the hippocampus with AP5.

However since latent extinction is a spatial form of extinction, the question arises that if an animal were to learn a task that can only be acquired using the habit memory system, would it be possible for that animal to extinguish that learning based solely on spatial information. The knowledge that a spatial location no longer predicts the location of the platform is useless if the animal did not pair a spatial location to the platform during initial acquisition. A single solution water maze task has also been developed where the animal can acquire the task only with stimulus-response habit learning. In this single solution response task, the animal is trained to locate a hidden platform whose location varied but the same body turn was reinforced regardless of starting position. If an animal was trained in this single solution response task, it is predicted in experiment

5b that while response extinction would be acquired normally, latent extinction would not be possible since the animal did not acquire any spatial information during initial task acquisition.

Method

Subjects

Subjects were 44 adult male Long-Evans rats (275-300 g). All animals received food and water *ad libitum*.

Apparatus

The water plus-maze described in the general methods is used for experiment 5.

Surgery

Animals received bilateral cannulation surgeries in the hippocampus as described in the general methods.

Drugs and Infusions

D-AP5 (Tocris) was dissolved in physiological saline and infused intra-hippocampally at a dose of either 5 µg/0.5µl or 7.5 µg/0.5µl. These doses were based on previous work from our lab examining the effect of intra-PFC infusions of AP-5 on conditioned place preference extinction (Hsu & Packard, 2008) Control animals were infused with physiological saline. Bilateral intra-hippocampal infusions were administered as described in the general methods.

Single-Solution Place-Task Acquisition Training

Animals were trained for five days at 6 trials/day to find a hidden platform located in a consistent location (i.e. west). Start locations are alternated as follows: NSSNNS on odd days and SNNSSN on even days. Once the animal located the hidden platform, it

remained there for 10 s and was then removed and placed in opaque holding box adjacent to the maze for a 30 second inter-trial interval. If the animal did not reach the hidden platform in 60s, it was guided there manually. On each trial both the latency (seconds) to reach the hidden platform and incorrect arm visits were recorded and used as a measure of task acquisition. An incorrect arm visit was defined as a full body length entering the arm not containing the hidden platform. This task is defined as a "single-solution" *place* task because rats can only use a place solution to solve the task since each body turn is equally reinforced, but the location of the hidden platform is consistent. Both latency to reach the hidden platform and errors were recorded and used as a measure of task acquisition.

Single-Solution Response-Task Acquisition Training

Training procedures were identical to those of the single solution place task with the exception that rather than the hidden platform being located in a consistent location, it was placed so that with each trial the location of the hidden platform varies, but the same body turn response (e.g. turn right) was reinforced (i.e. platform is located in the "west" arm when the animal is started in the "north" arm and located in the "east" arm when the animal is started in the "south" arm). This task is defined as a "single-solution" *response* task because rats can only use a response solution to solve the task since platform locations are equally reinforced, but the location of the hidden platform is consistent. Both latency to reach the hidden platform and errors were recorded and used as a measure of task acquisition.

Extinction Training: General Procedure

Twenty-four hours following the completion of acquisition training (i.e. day 6), rats were matched based on latencies to reach the platform during the last three days of acquisition to form a total of five extinction groups in the single-solution place-task ; three "latent" extinction groups (5 µg AP5, n = 9; 7.5 µg AP5, n = 7; and saline, n = 10), and two "response" extinction groups (7.5 µg AP5, n = 7; and saline, n = 6) and two extinction groups in the single-solution response-task, latent extinction and response extinction (n=6 per group). For both the response and latent conditions, extinction training was administered over two days (6 trials/day, 30 second inter-trial interval), and rats received intra-hippocampal infusions of saline or AP5 immediately prior to extinction training on each of these two days.

Latent Extinction Training

In the latent extinction condition, rats were placed by the experimenter in the location previously occupied by the hidden platform and were confined for 20 seconds by placement of a clear Plexiglas shield (10.5 inches from the end of the maze arm). Following confinement, rats were removed from the maze and placed in an opaque holding box located on a table adjacent to the maze for a 30 second inter-trial interval.

Response Extinction Training

In the response extinction condition, rats were placed into the start end of the maze as during training and allowed to swim in the maze without the platform present. Upon reaching the end of the goal arm and either remaining in the goal arm for 10s or attempting to enter another arm (or after 60 seconds if the rat does not reach the end of the goal arm), rats were removed from the maze and placed in an opaque holding box

located on a table adjacent to the maze for a 30 second inter-trial interval. Both latency to reach the end of the goal arm and errors were recorded and used as a measure of extinction behavior.

Extinction Testing

Twenty-four hours following the completion of extinction training (i.e. day 8), rats were given four extinction "probe" trials in which they were placed in the start arm and allowed to swim as in acquisition with starting locations varying at: SNNS. Both latency to reach the end of the goal arm and errors are recorded and used as a measure of extinction behavior. The hidden platform was not present for the probe trials.

Control Experiment

An additional group of animals (n = 9) received maze training in the single solution place task. However, instead of extinction in the maze, the animals were confined to a bucket filled with water for 2 days the equivalent amount of time as in latent extinction training (6 trials/day, 20s confinement per trial). 24 hours following the second "extinction" day, the animals received four "probe" trials as described above. This experiment was designed to verify that decreased latencies following latent extinction are not due to a learned helplessness effect.

Results

Experiment 5a: Acquisition of Single-Solution Place-Task Maze Behavior

A two-way one-repeated measures ANOVA (Group X Trial) computed on the latencies to reach the hidden platform during acquisition revealed a non-significant interaction ($F_{8,23} = 0.715$, n.s.] and a non-significant main effect of Group ($F_{2,23} = 2.431$, n.s] for the acquisition of the single-solution place-task for the rats that *subsequently*

received saline or AP5 (5.0 ug or 7.5 ug) prior to latent extinction training A significant Trial effect ($F_{4, 23} = 54.194$, $p < 0.001$) revealed that the latency to reach the hidden platform improved in all three groups at a similar rate.

A two-way one-repeated measures ANOVA (Group X Trial) computed on the latencies to reach the hidden platform during acquisition revealed a non-significant interaction ($F_{4,11} = 0.915$, n.s.) and a non-significant main effect of Group ($F_{1, 11} = 0.115$, n.s.) for the acquisition of the single-solution place-task for the rats that *subsequently* received saline or AP5 (7.5ug, 5 ug) prior to response extinction training. A significant Trial effect ($F_{4, 11} = 19.077$, $p < 0.001$) revealed that the latency to reach the hidden platform improved in both groups at a similar rate. Thus, any differences in behavior between saline and AP5 groups during latent or response extinction testing are not due to differential rates of initial task acquisition.

Experiment 5a: Latent and Response Training Produce Significant and Comparable Extinction

Several analyses were conducted in order to examine whether response and latent extinction training used resulted in significant and comparable levels of extinction in control (i.e. saline-treated) rats. The acquisition and extinction of the single-solution place-task for the rats that *subsequently* received latent or response extinction training is shown in Figure 5.1. First, a one-way ANOVA was conducted comparing the mean latency to reach the location of the hidden platform on the final block of acquisition trials and the probe trials. The analysis revealed a significant difference in both the response (last acquisition M = 4.167, SEM = 0.538, probe M = 21.250, SEM = 11.434; $F_{1,10} = 13.363$, $p < .01$), and latent (last acquisition M = 4.190, SEM = 1.166, probe M = 18.571,

SEM = 9.186; $F_{1,12} = 16.884$, $p < .001$) extinction control groups, indicating that both types of extinction training resulted in significant extinction of swim behavior in control rats.

An additional measure of the effectiveness of latent extinction in control rats was provided by comparing the mean latency to reach the location of the hidden platform on the four probe trials (M = 18.571, SEM = 9.186) with the mean latency on day one (trials 1-4) of rats receiving response extinction training (M = 9.042 , SEM = 3.523) . A one-way ANOVA revealed a significant group difference ($F_{1,11} = 5.678$, $p < .05$). This finding indicates that the latencies of control rats that had received latent extinction training were significantly higher on the four probe trials than those of control rats receiving their initial four trials of response extinction training. Therefore, the latent training resulted in extinction behavior that cannot be accounted for simply by the non-rewarded swimming responses that occurred over the four probe trials.

A final analysis of the behavior of control rats was conducted in order to compare the overall *relative* effectiveness of the response and latent extinction training. One-way ANOVA's comparing the mean latency to reach the location of the hidden platform for the final set of four extinction probe trials revealed no significant difference between control rats trained in the response (M = 21.250, SEM = 11.434) and latent (M = 18.571, SEM = 9.186) extinction conditions ($F_{1,11} = 0.220$, n.s.). This finding suggests that the response and latent training procedures resulted in comparable levels of extinction. Thus, any selective behavioral effect of AP5 cannot be due to a difference in task difficulty between the two types of extinction training.

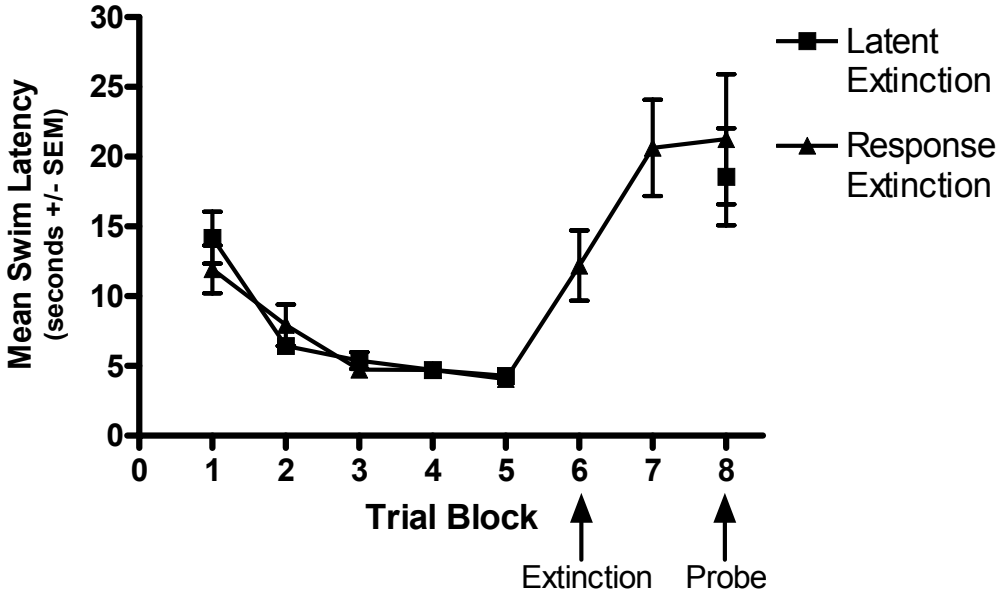

Figure 5.1 The effect of latent and response extinction in the single solution place task (experiment 5). Mean (+ SEM) of latency (in seconds) to reach the hidden platform over trial block by group. Latent and response extinction produce comparable levels of extinction in the single solution place task.

Experiment 5a: Intra-Hippocampal AP5 Impairs Latent Extinction in a Dose Dependent Manner

The effect of intra-hippocampal infusions of saline (M = 16.550, SEM = 8.957) or AP5 (7.5ug - M = 7.643, SEM = 4.264; 5ug - M = 12.781, SEM = 6.702) on latent extinction is shown in Figure 5.2. A one-way ANOVA computed on the mean latency to reach the location of the hidden platform for the four extinction probe trials revealed a significant Group difference ($F_{2,22} = 3.920$, $p < .05$), indicating that dorsal hippocampal inactivation blocked latent extinction. Post-hoc analyses revealed that the AP5 dose of 7.5ug/0.5ul effectively blocked latent extinction ($p < .05$) while the AP5 dose of

5.0ug/0.5ul did not.

An additional measure of the effectiveness of 7.5ug AP5 in blocking latent extinction was provided by comparing the mean latency to reach the location of the hidden platform on the four probe trials (M = 7.643, SEM = 4.264) with the mean latency of control rats on day one of response extinction training (9.042, SEM = 3.523). This comparison was conducted to determine whether 7.5ug AP5-treated rats receiving latent extinction performed similar to control rats during the initial response extinction training. A one-way ANOVA comparing the mean latency to reach the location of the hidden platform over the four probe trials in 7.5 ug AP5-treated rats that had received latent extinction, and the mean latency for control rats on trials 1-4 on day one of response extinction revealed no significant difference ($F_{1,12} = 0.406$, n.s.). Consistent with a drug-induced blockade of latent extinction, this finding indicates that the latencies of AP5-treated rats on the four probe trials were similar to those of control rats receiving the initial four trials of response extinction training.

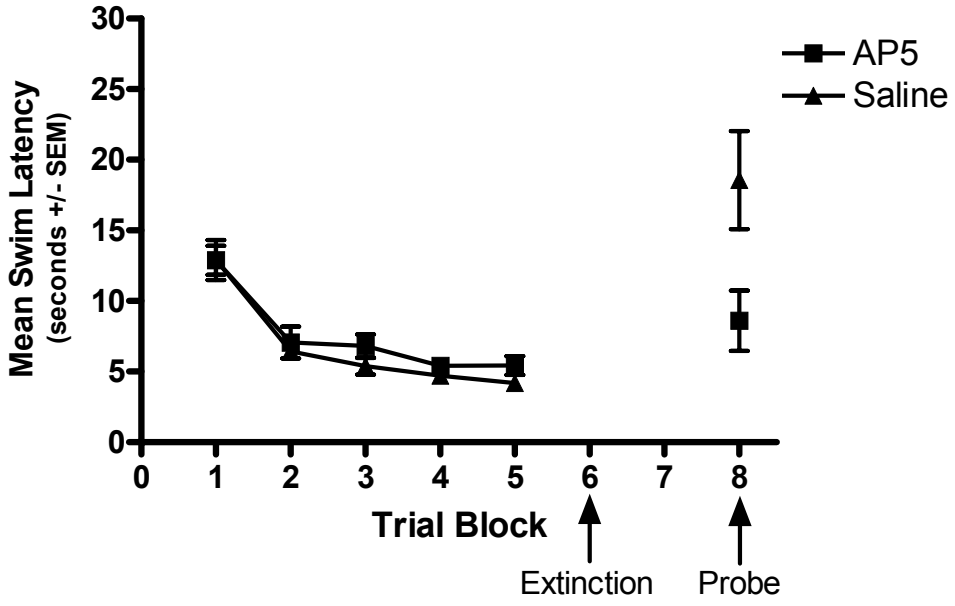

Figure 5.2 The effect of intra-hippocampal AP5 (7.5 ug/0.5 ul) on latent extinction in the single solution place task (experiment 5). Mean (+ SEM) of latency (in seconds) to reach the hidden platform over trial block by group. Intra-hippocampal AP5 impaired latent extinction.

Experiment 5a: Intra-Hippocampal AP5 Does Not Impair Response Extinction

The effect of intra-hippocampal infusions of saline or 7.5ug AP5 on response extinction is shown in Figure 5.3. A two-way one-repeated measures ANOVA (Group X Trial) computed on the latencies to reach the location of the hidden platform during response extinction training revealed a non-significant interaction ($F_{1,11} = 0.023$, n.s.) and a non-significant main effect for Group ($F_{1,11} = 0.483$, n.s.). A significant Trial effect ($F_{1,11} = 15.944$, $p < .01$), revealed that latency to reach the location of the hidden platform increased at a similar rate in both groups during the two days of response extinction. In addition, a one-way ANOVA computed on the overall mean latency to

reach the location of the hidden platform on the subsequent four extinction "probe" trials revealed a non-significant Group difference ($F_{1,12} = 0.118$, n.s.) between groups receiving AP5 (M = 23.643, SEM = 13.324) and controls (M = 21.250. SEM = 11.434), further indicating that 7.5ug AP5 did not block response extinction.

Figure 5.3 The effect of intra-hippocampal AP5 (7.5 ug/0.5 ul) on response extinction in the single solution place task (experiment 5). Mean (+ SEM) of latency (in seconds) to reach the hidden platform over trial block by group. Intra-hippocampal AP5 did not impair response extinction.

Latent Extinction Is Not the Result of Learned Helplessness in the Water Maze

In a control study run to determine whether the latent extinction effect can be attributable to learned helplessness following confinement in the water maze, a one way ANOVA comparing the mean latency to reach the location of the hidden platform on the four probe trials for animals that received "latent extinction" in the bucket (M = 7.740, SEM = 3.377) with the mean latency of control rats on day one of response extinction

training (9.042, SEM = 3.523) revealed no significant differences ($F_{1,12} = 0.491$, n.s.) demonstrating that confinement to a bucket during the extinction period did not result in a latent extinction effect (Figure 5.4). An additional comparison was made between the mean latency to reach the location of the hidden platform on the four probe trials animals that received latent extinction on the maze (M = 18.571, SEM = 9.186) to those that received latent placements in the bucket (M = 7.740, SEM = 3.377). A one-way ANOVA revealed a significant group difference ($F_{1,13} = 9.714$, $p < 0.01$). These findings demonstrate that confinement to a bucket is not sufficient to induce an extinction effect and that latent extinction in the water maze cannot be attributed to learned helplessness.

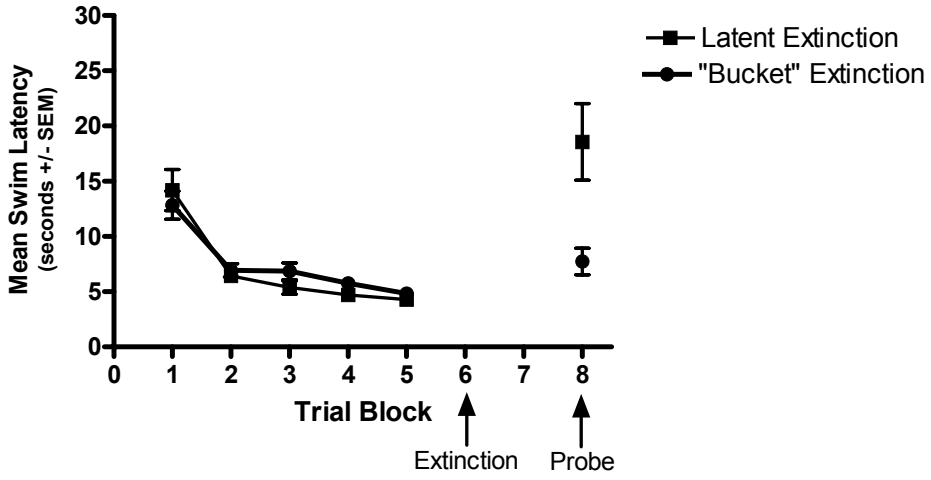

Figure 5.4 The effect of bucket confinement during the latent extinction period in the single solution place task (experiment 5). Mean (+ SEM) of latency (in seconds) to reach the hidden platform over trial block by group.

Experiment 5b: Acquisition of Single-Solution Response-Task Maze Behavior

The acquisition of single-solution response-task for the rats that *subsequently* received latent or response extinction training is shown in Figure 5.5. A two-way one-

repeated measures ANOVA (Group X Trial) computed on the latencies to reach the hidden platform during acquisition revealed a non-significant interaction ($F_{4,10} = 0.235$, n.s.) and a non-significant main effect of Group ($F_{1,10} = 0.447$, n.s). A significant Trial effect ($F_{4, 10} = 19.076$, $p < 0.001$) revealed that the latency to reach the hidden platform improved in both groups at a similar rate. Thus, any differences in behavior between extinction groups during latent or response extinction testing are not due to differential rates of initial task acquisition.

Experiment 5b: Latent Extinction Training Does Not Produce Comparable Extinction to Response Extinction Training Following the Single-Solution Response-Task

Several analyses were conducted in order to examine whether response and latent extinction training used resulted in significant and comparable levels of extinction in control (i.e. saline-treated) rats. First, a one-way ANOVA was conducted comparing the mean swim latency on the final block of acquisition trials and the final block of extinction trials. The analysis revealed a significant difference in both the response ($F_{1,11} = 40.691$, $p< .001$), and the latent ($F_{1,11} = 15.120$, $p < .01$] extinction control groups, indicating that both types of extinction training resulted in significant extinction of swim behavior in control rats. However, in a comparison of the mean latency to reach the location of the hidden platform on the four probe trials of the rats receiving latent extinction training ($M = 9.750$, $SEM = 2.683$) with the mean latency on extinction day one (trials 1-4) of rats receiving response extinction training ($M = 13.125$, $SEM = 7.926$) did not revealed a significant group difference ($F_{1,11} = 0.976$, n.s.). This finding indicates that the significant extinction effect of following latent extinction training can

be accounted for simply by the non-rewarded swimming responses that occurred over the four probe trials.

A final analysis of the behavior of control rats was conducted in order to compare the overall *relative* effectiveness of the response and latent extinction training. One-way ANOVA's comparing the mean latency to reach the location of the hidden platform for the four extinction probe trials revealed a significant difference between control rats trained in the response (M = 30.333, SEM = 9.885) and latent (M = 9.750, SEM = 2.683) extinction conditions ($F_{1,11} = 24.229$, $p < 0.01$]. This finding reveals that the response and latent training procedures did not result in comparable levels of extinction.

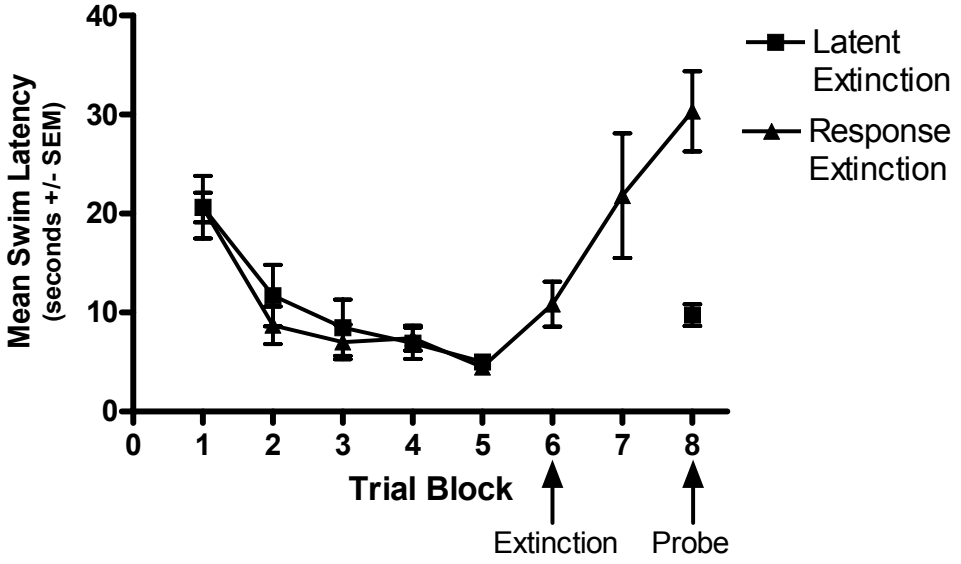

Figure 5.5 The effect of latent and response extinction in the single solution response task (experiment 5). Mean (+ SEM) of latency (in seconds) to reach the hidden platform over trial block by group. Latent extinction is not effective following acquisition in the single solution response task.

Discussion

Single-Solution Place Task Learning Can be Extinguished with Both Response and Latent Extinction

The present studies examined whether requiring a specific type of learning during task acquisition can affect the types of learning that can be acquired during extinction. Experiment 5a indicated that learning in the single-solution place task can be extinguished with either latent or response extinction. Since this task is a hippocampus-dependent spatial task (Schroeder, Wingard, & Packard, 2002), it follows that this learning could be extinguished with a spatial form of extinction. Additionally, since response extinction is acquired in the same manner as initial task acquisition, response extinction was also expected to be effective. These results indicate that the performance of the previously acquired response during extinction is not necessary for extinction in the water maze task.

Intra-hippocampal AP5 Impairs Latent Extinction of the Single-solution Place Task

Previous studies from our lab have shown latent extinction in the straight alley to be both hippocampus-dependent (Gabriele & Packard, 2006) and NMDA receptor mediated (Gabriele & Packard, 2007, experiment 4). The current study also found that latent extinction of the single-solution place task was impaired following intra-hippocampal administration of AP5, indicating that hippocampal NMDA receptors play a role in latent extinction. These results are consistent with previous findings indicating that AP5 impairs hippocampal dependent learning in several tasks (Packard & Teather, 1997; Steele & Morris, 1999; Liang, et. al., 1994; Yoshihara & Ichitani, 2004; Wanisch, et. al., 2005) however; these are the first to demonstrate an impairment following AP5

administration in extinction where the overt response is no longer required. Response extinction of the single-solution place task was not impaired following intra-hippocampal AP5 administration, demonstrating that hippocampal NMDA receptor activation is not necessary when there is a response component to the extinction. While this task can only be acquired with a spatial strategy and thus can be extinguished the same way, it is possible that, without the hippocampus available, the animal can still learn that a response is no longer effective at locating the hidden platform during extinction and instead attempt a new response. This learning does not necessarily have to be spatial in nature. Taking a multiple memory systems perspective, other brain structures such as the dorsal striatum, which is implicated in S-R learning (for review see Packard, 2001; McDonald & White, 1994) and response extinction in a straight alley maze (experiment 1), may contribute to the acquisition of this response component and allow the animal to extinguish normally. However, it remains to be examined whether other tasks that are only acquired spatially can be extinguished with multiple learning systems such as caudate mediated stimulus-response habit system.

Latent Extinction Is Ineffective in the Single-solution Response Task

The question addressed by experiment 5b is whether *requiring* the use of a specific memory system during acquisition affects the ability to use a different system during extinction. While the single-solution place task can be extinguished with either latent or response extinction, this was not the case for the single-solution response task. Following acquisition in the single-solution response task, response extinction was effective but latent extinction was impaired. The impairment of latent extinction indicates that by requiring habit learning during acquisition, cognitive extinction can be rendered

ineffective. When the animal learned the single-solution response task, the only effective strategy was to learn a consistent body-turn response. Spatial information is irrelevant, and even an impairment in solving this task. Subsequently, the animal is not acquiring spatial information about the location of the hidden platform during task acquisition. Therefore, the knowledge that a particular spatial location no longer contains the hidden platform is ineffectual. The animal only learns a consistent body-turn response, and since this response is not performed during latent extinction, the response is not extinguished. The previous experiment using the single-solution place task indicated that latent extinction is effective at extinguishing the swimming response following place learning during acquisition, so this impairment in latent extinction is habit-task specific. These results are consistent with previous research showing that the hippocampus is not necessary for extinction of S-R habit tasks (Niki, 1962; Thomas & McCleary, 1974; Kaplan, 1968; Nadel, 1968; Gaffan, 1972), however these studies are the first to show that requiring a specific type of learning during acquisition can influence the relative use of different types of learning during extinction. In tasks that can be acquiring using either cognitive or habit memory systems simultaneously and in parallel, spatial information is acquired early in training and the slower incremental learning of the habit S-R system prevails later in training (McDonald & White, 1994; Packard & McGaugh, 1996; Packard, 1999). The question remains as to whether cognitive extinction would be affected once learning has transitioned to habit memory, even though spatial information was previously acquired. Latent extinction is impaired following extended training in a straight alley (Dyal, 1963; Clifford, 1964) and perhaps an explanation is that this learning has transitioned to habit thus impairing cognitive extinction.

CHAPTER VIII

EXPERIMENT 6

Introduction

Understanding the neural mechanisms underlying both the acquisition and extinction of drug addiction has important clinical implications for treatment therapies targeting drug addiction. Applying a multiple memory systems approach to the study of extinction behavior may be beneficial for the development of treatments for the extinction of maladaptive behavior. The acquisition and extinction of learned behavior involves multiple memory systems (Cohen & Squire, 1980; Eichenbaum & Cohen, 2001; Hirsh, 1974; Mishkin & Petri, 1984; O'Keefe & Nadel, 1978; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002; Gabriele & Packard, 2006) and in several tasks the hippocampal and caudate systems are activated simultaneously and in parallel. When in competition, the cognitive based hippocampal system prevails early in training, while later in training the habit based striatal system takes over (McDonald & White, 1994; Packard & McGaugh, 1996; Packard, 1999). Similar to the acquisition of many learned behaviors, the acquisition of drug addiction potentially involves a shift from goal directed actions to compulsive drug seeking behavior. Specifically, during drug seeking a transition may occur from the ventral striatum to the dorsal striatum indicating that responding for drug reward become habitual over time (Everitt & Robbins, 2005; Haber et al., 2000; Porrino et al., 2004; See et al., 2007; Belin & Everitt, 2007). While this switch may mirror the switch from cognitive to habit learning in normal behavior, it is believed that during the acquisition of drug addiction, the habit systems may be "usurped" into a more maladaptive form of habit based behavior (for review see Everitt et

al, 2001; Everitt & Robbins, 2005; White, 1996). Perhaps during the acquisition of drug addiction, this switch to habit learning occurs more rapidly and more strongly than in normal behavior.

Previous research has shown that requiring the use of a stimulus-response learning strategy during acquisition renders a spatial-cognitive strategy ineffective during extinction (experiment 5.2). Because requiring habit learning during acquisition prevents spatial extinction, perhaps the strong habit-like compulsion believed to be acquired during drug addiction (for review see Everitt et al, 2001) may affect the types of memory systems available to extinguish this addiction. In the present study, we examined whether cognitive latent extinction is affected following acquisition of drug seeking.

The oral cocaine model was used in this study for several reasons. Using an oral cocaine solution as a reward in a maze task allows the animal to actively engage in drug seeking behavior and self-administer without experimenter interference. Additionally, this model more appropriately mirrors human drug seeking behavior in that the rat both actively seeks out and self-administers the drug. Animals will readily self administer an oral cocaine solution (Falk et al., 1990; Jentsch et al., 1998; Suzuki et al., 1990; Miles et al., 2003)and oral cocaine produces drug dependence in that it produces withdrawal following forced abstinence (Barros & Miczek, 1996) and additionally is resistant to reinforcer devaluation unlike a control solution of natural reinforcers (Miles et al, 2003). For these reasons, we felt the oral cocaine model was the most effective model to examine our theoretical questions.

Experiment 6 aims to examine the effect of oral cocaine administration during acquisition on latent and response extinction to address how drug use affects the relative

use of multiple memory systems during extinction within the context of active drug seeking behavior.

Method

Subjects

Subjects were 32 adult male Long-Evans rats (275-300 g). All animals received food *ad libitum*.

Apparatus

The straight alley maze described in the general methods is used for experiment 6.

Drugs

During maze training, animals received either a cocaine-sucrose solution (0.1% cocaine HCl, 20% sucrose) or a sucrose solution (20%) reward. Each animal received 0.5 mls of solution per trial at 6 trials/day for a total volume of 3 mls/day. Given a 300g rat, this is equivalent to a 10 mg/kg dose.

Solution Habituation

Water bottles were removed from home cages 24 hours prior to solution habituation and animals received 15 min per day access to water bottles throughout training. Each animal received habituation training to the solution (sucrose or cocaine) to be received in maze training. Habituation consisted of 3 consecutive days of presentation of 0.5 mls of the solution in a novel environment, with the number of exposures increasing with each habituation day (1, 2, 4) in order to prepare the animals to drink the reward solution over multiple trials in the maze environment. Volume consumed and amount of time to consume the solution were recorded for each animal. Each sucrose animal was matched to a cocaine animal in terms of volume of solution delivered during

habituation to ensure that there were no differences between groups in terms of volume of solution consumed prior to training.

Straight-Alley Maze Acquisition Training

Straight alley maze acquisition training was identical to the methods described in experiment 1 with the exception that the reward was switched from a food reward to a solution reward.

Extinction Training: General Procedure

Twenty-four hours following the completion of acquisition training (i.e. day 11), rats were assigned one of two extinction conditions; "response" extinction (n = 14) and "latent" extinction (n = 18). Both latent and response extinction were identical to the methods described in experiment 1.

Extinction Testing

Extinction testing procedures were identical to those described in experiment 1.

Results

Latent Extinction

A one-way ANOVA indicated significant difference in latent extinction across probe trials for those animals receiving an oral cocaine reward during acquisition (M = 14.450, SEM = 9.552) as compared to those receiving a sucrose reward (M = 25.375, SEM = 9.791) across probe trials ($F_{1,16} = 5.688$, $p < 0.05$) indicating a significant impairment of latent extinction (Figures 6.1, 6.2). Further analyses revealed that there was a significant difference between the last day of acquisition training and the probe trails for both animals that received cocaine (last acquisition M = 3.916, SEM = 2.410; probe M = 14.450, SEM = 9.552) ($F_{1,18} = 11.435$, $p < 0.005$) and sucrose (last acquisition

M = 7.043, SEM = 6.371; probe M = 25.375, SEM = 9.791) ($F_{1,14}$ = 19.705, p <0.001) indicating that both groups displayed a significant extinction effect. Additionally, in a two-way one-repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,16}$ = 61.030, p < 0.001) indicating significant differences in latencies between days. There was not a significant main effect for solution reward ($F_{1,16}$ = 1.937, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and solution reward ($F_{9,16}$ = 0.529, n.s.) indicating that both groups acquired the task at a similar rate.

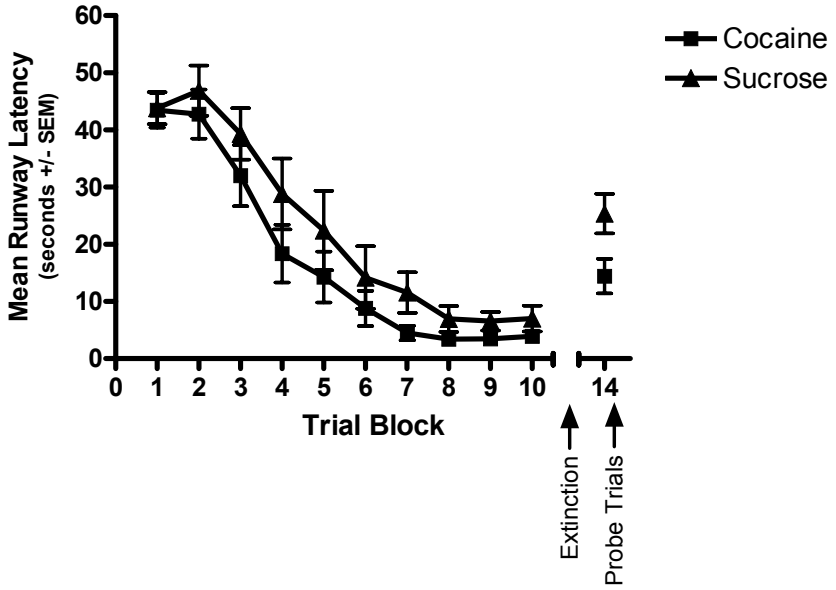

Figure 6.1 The effect of oral cocaine use during acquisition on latent extinction (experiment 6). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Oral cocaine use impaired latent extinction as compared to sucrose controls.

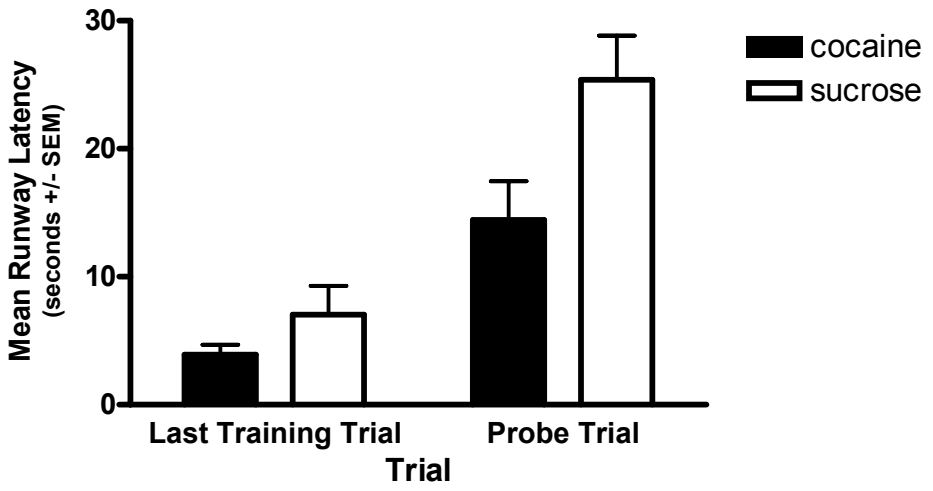

Figure 6.2 Latent extinction following oral cocaine use during acquisition (experiment 6). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Oral cocaine use impaired latent extinction as compared to sucrose controls.

Response Extinction

A one-way ANOVA indicated no difference in response extinction across probe trials for those animals those animals receiving an oral cocaine reward during acquisition (M = 41.610, SEM = 9.222) as compared to those receiving a sucrose reward (M = 37.429, SEM = 9.967) ($F_{1,12}$= 0.663, n.s.) (Figures 6.3, 6.4). Additionally, a two-way one-repeated measures ANOVA indicated no group differences during acquisition of response extinction, with a significant main effect for extinction day ($F_{2, 12}$ = 16.437, p < 0.001), but no significant effect for solution reward ($F_{1, 12}$ = 2.269, n.s.) and no significant interaction between extinction day and solution reward ($F_{2,12}$ = 0.878, n.s.) were found. Also, there was a significant difference between the last day of acquisition training and the probe trails for both animals that received cocaine (last acquisition M = 4.528, SEM = 4.239; probe M = 41.610, SEM = 9.222) ($F_{1,12}$ = 93.457, p < 0.001) and

sucrose (last acquisition M = 10.547, SEM = 11.622; probe M = 37.429, SEM = 9.967) ($F_{1,12} = 21.578$ $p < 0.005$) indicating that both groups displayed a significant extinction effect. Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{9,12} = 13.107$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1,12} = 0.438$, n.s.) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{9,12} = 1.503$, n.s.) indicating that the task was acquired at a similar rate for both groups.

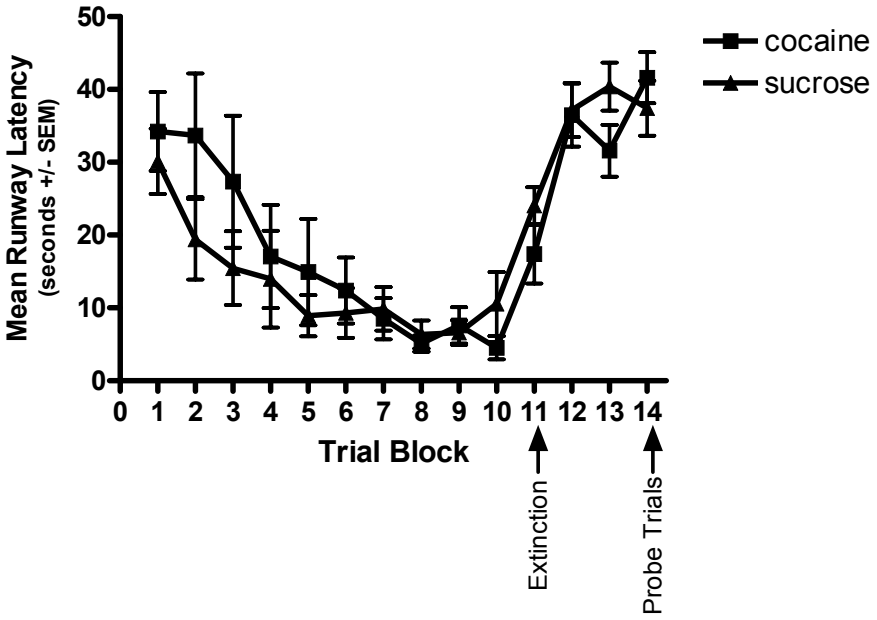

Figure 6.3 The effect of oral cocaine use during acquisition on response extinction (experiment 6). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Oral cocaine use did not impair response extinction as compared to sucrose controls.

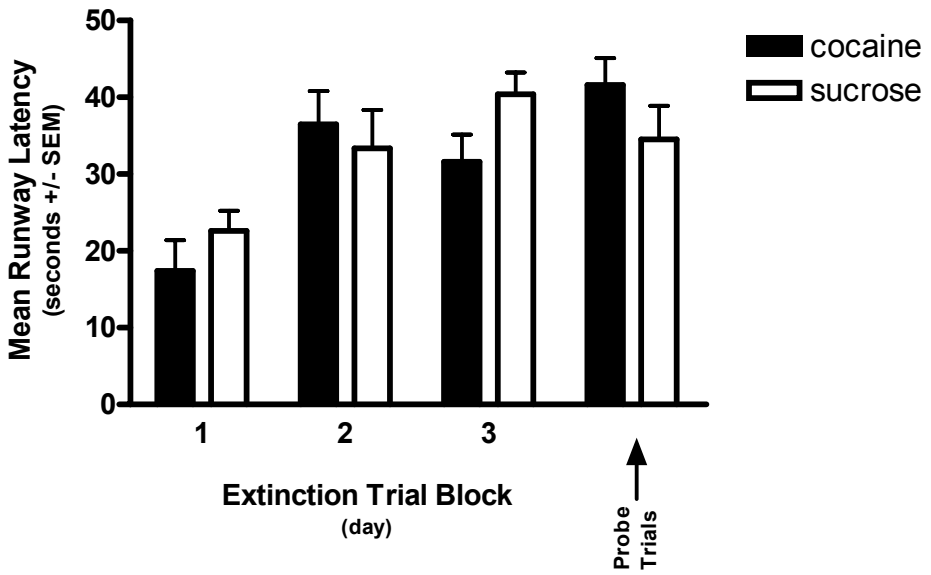

Figure 6.4 Response extinction following oral cocaine use during acquisition (experiment 6). Mean (+ SEM) of latency (in seconds) to reach the goal over trial block by group. Oral cocaine use did not impair response extinction as compared to sucrose controls.

Discussion

The present experiments investigated the effect of oral cocaine use on the relative use of different extinction strategies in a straight alley maze. Following solution acquisition training, rats in the response extinction condition performed the approach response to an empty goal box while rats in the latent extinction were confined in the goal box with no reward present. Consistent with previous food rewarded studies (Gabriele & Packard, 2006; Seward & Levy, 1949) animals rewarded with a sucrose solution were able to extinguish the approach response with or without performing the previously rewarded response during extinction training. However, animals rewarded with a cocaine

solution showed normal response extinction, but were significantly impaired in acquiring latent extinction.

The differences in effects between drug groups cannot be explained due to a difference in locomotor activity due to the fact that there were no differences in initial task acquisition between groups. Additionally, the animals rewarded with the cocaine solution extinguished normally in the response condition and did not show any locomotor enhancement compared to the sucrose rewarded group that could potentially explain the increased latencies following latent extinction training in the cocaine rewarded group. These results indicate that cocaine use has the ability to affect the relative use of multiple memory systems during extinction learning. Since the acquisition of the straight alley task can be acquired by either memory system, these differences would only be apparent during extinction. From a multiple memory systems perspective, this impairment in hippocampus-dependent "cognitive" latent extinction can be explained two ways. Either cocaine use impairs hippocampal dependent spatial learning or enhances striatal dependent habit learning. Since the acquisition of drug addiction may involve a distinct transition from goal directed behavior to a compulsive habit, and it is proposed that this compulsive drug use may be the result of a maladaptive functioning of this transition (for review see Everitt et al, 2001; Everitt & Robbins, 2005; White, 1996), both possibilities should be considered.

This switch to habitual learning that potentially characterizes addiction may occur through the 'spiraling' loop circuitry in the striatum, in which information progresses in a ventral to dorsal pattern throughout the striatum (Haber et al, 2000). Previous studies examining instrumental responding for food reward have shown that stimulus-response

learning is mediated by the dorsal striatum (Graybiel, 1998; McDonald & White, 1993; Packard & Knowlton, 2002; Knowlton et al., 1996; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002). If the compulsive drug use of an addict is the action of a maladaptive form of normal stimulus-response learning then dorsal striatum could be responsible for this behavior.

Recent evidence has implicated the dorsal striatum in habitual drug seeking behavior (Everitt & Robbins, 2005; Haber et al., 2000; Porrino et al., 2004; See et al., 2007; Belin & Everitt, 2007). Extended drug use results in altered functional activity in the dorsal striatum (Porrino et al., 2004) and dopamine increases in the dorsal striatum are seen following presentation of a contingent cue associated with cocaine (Ito et al., 2002). Additionally, inactivation of the dorsal striatum attenuates drug seeking following both abstinence and extinction following extended drug use (Fuchs et al., 2006; See et al., 2007) indicating that the dorsal striatum may even be necessary for the habit-based component of compulsive drug seeking.

Prior to habitual drug seeking, drug use is motivated by the desire to experience the rewarding effects of the drug (Robbins & Everitt, 2002). Since the hippocampus is involved in early learning of acquired behaviors (McDonald & White, 1994; Packard & McGaugh, 1996; Packard, 1999), it is possible that the hippocampus may contribute to early learning of drug seeking behaviors in terms of declarative memory used to learn about the environment in which drug taking occurs and associate it with the drug use itself (White, 1996). Several recent studies have demonstrated that cocaine pre-exposure causes impairments in hippocampal-dependent tasks such as the Morris water maze and the win-shift radial arm maze task (Quirk et al., 2001; Melnick et al, 2000; Mendez et al.,

2008; but see also Del Olmo et al, 2006) indicating that drug use may impair the hippocampal memory system.

The present results indicate that oral cocaine influences the relative effectiveness of multiple memory systems during extinction. The dorsal striatum has been found to play a role in cue induced drug seeking (Ito et al, 2002, Garavan et al, 2000, Vanderschuren et al, 2005), while the hippocampus may be impaired during early learning of drug seeking behavior (Quirk et al, 2001, Melnick et al, 2000; Mendez et al., 2008). This indicates that the potentially maladaptive form of habitual learning that occurs during drug seeking may involve a dysfunction of the normal transition from the hippocampal to striatal memory system.

CHAPTER IX

CONCLUSIONS

Summary of Results

The present studies examined the neuroanatomical and neurochemical bases of latent extinction with the intent to further understand the neural bases of extinction behavior and provide some applications for the use of latent extinction in various extinction therapies. In the experiments completed in Aim 1, following food rewarded training in a straight alley maze, animal were given either latent or response extinction and subsequent extinction behavior was assessed. Reversible neural inactivation of the dorsolateral caudate prior to extinction selectively attenuated response extinction, while latent extinction remained intact. These results, coupled with our previous findings demonstrating a selective impairment of latent extinction following dorsal hippocampal inactivation (Gabriele & Packard, 2006) establish a double dissociation of extinction behavior for these two brain structures and provide further evidence that the learning that underlies extinction involves multiple memory systems. Further, inactivation of either the basolateral amygdala or the medial prefrontal cortex did not affect the acquisition of either form of extinction under the current training parameters further demonstrating the selective involvement of the hippocampal and caudate memory systems in latent and response extinction, respectively. However it remains to be seen whether the amygdala or prefrontal systems, while not necessary, are involved in the extinction of runway behavior or if further behavioral manipulations can recruit either of these systems.

Additional studies completed in Aim 2 examining the neurochemical basis established a role for the glutamatergic system in latent extinction. Peripheral NMDA

receptor agonism with D-cycloserine enhanced latent extinction in the straight alley maze and intra-hippocampal NMDA receptor antagonism with AP-5 selectively blocked latent extinction in a water plus maze task demonstrating a role for hippocampal NMDA receptors in the acquisition of latent extinction.

Further experiments from Aim 2 demonstrated that, following acquisition in a water maze single-solution place task which is preferentially acquired with spatial learning, both latent and response extinction are effective. However, following acquisition in the water maze single-solution response task which is preferentially acquired with stimulus-response habit learning, response extinction was effective but latent extinction was impaired. These results indicated that requiring the use of a specific memory system during acquisition can affect the relative use of multiple memory systems during extinction.

Given that the memory system activated during acquisition can affect the memory systems available during extinction, Aim 3 investigated whether cocaine use, which may subvert normal mechanisms of acquisition of stimulus-response habits (for review see Everitt et al., 2001) would affect the relative use of multiple memory systems during extinction. Animals trained on the straight alley maze for an oral cocaine-sucrose reward showed impaired latent extinction as compared to controls trained to run for a sucrose alone reward indicating that cocaine use impairs extinction learning under conditions in which new stimulus-response learning is prevented.

Taken as a whole, the present results provide a further understanding of the neuroanatomical and neurochemical bases of latent extinction. Further, these findings provide significant evidence that multiple forms of memory underlie extinction behavior,

which has potential implications for the development of more effective clinical extinction therapies.

What Is Learned During Extinction?

While recent evidence has clearly demonstrated that there is "more than one type of learning" (Tolman, 1949) (Cohen & Squire, 1980; Eichenbaum & Cohen, 2001; Hirsh, 1974; Mishkin & Petri, 1984; O'Keefe & Nadel, 1978; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002; Zola-Morgan & Squire, 1984; Scoville & Milner, 1957; Knowlton, et al., 1996) this theory has not been applied to extinction behavior. One current extinction theory states that extinction involves the formation of an inhibitory stimulus-response association (Rescorla, 1993a; 1993b; 1996; 1997; 2001; Delamater, 1996; 2004). Much of the work investigating the type of associations that mediate extinction has been done by Rescorla who contends that response-outcome associations are "relatively impervious to modification," (pg. 244, 1993a) and therefore are maintained during extinction. This was demonstrated in an experiment in which rats were trained to make four instrumental individual responses resulting in two different types of reinforcers (food pellet and sucrose solution). Within a given training session, responses were consistently paired so that one response resulted in food while the other response resulted in sucrose. During the extinction session, only one pair of responses was extinguished therefore each reinforcer was paired with an extinguished response and a non-extinguished response. Following extinction, one of the reinforcers was devalued with a treatment of lithium chloride. At the extinction test in which both pairs of responses were tested, animals responded less with the pair that had been extinguished. However, within the extinguished pair of responses, animals responded less for the

response that had been paired with the devalued reinforcer indicating that the response-outcome association had been maintained throughout extinction (Rescorla, 1993a). Similar reinforcer devaluation studies have demonstrated that stimulus-outcome associations are also maintained following extinction (Rescorla, 1996). Rescorla argues that if both the response-outcome and stimulus-outcome associations are maintained then the decrement in responding seen in extinction is instead the act of an inhibitory stimulus-response association since animals learn hierarchical information about the stimuli, response, and outcomes associated with conditioning in the form of S-(R-O) (Rescorla, 2001). When an animal is trained to associate two different stimuli (e.g. light and tone) with food reward and then separately trained to perform two responses (R1 and R2) for food reward, the stimuli and responses are never presented together during acquisition in order to prevent any excitatory S-R associations from forming due to reinforcement. However, when both of the responses are extinguished, each in the presence of only one of the previously reinforced stimuli (e.g. R1 is extinguished in the presence of the light and R2 is extinguished in the presence of the tone), an inhibitory S-R association forms. Therefore, at the extinction test, responding in the presence of the stimulus which that particular response had been paired with during extinction was significantly less than in the presence of the stimulus that had not been paired with extinction. These results indicate that the stimulus had developed an inhibitory power that was specific to the response it had been paired with during extinction. These findings cannot be explained by alteration in the response-outcome association since those changes would affect both responses equally (Rescorla, 1993b). While these findings provide compelling evidence that R-O associations are maintained during extinction and that extinction involves the

formation of an inhibitory S-R association during instrumental tasks, the present results of this dissertation indicate that this explanation cannot account for all extinction situations. Arguments concerning the inadequacy of the fractional anticipatory response mechanism as an explanation of latent extinction (Gleitman et al., 1954; Treisman, 1960) indicate that it is unlikely an S-R association occurs during latent extinction. Additionally, previous research has demonstrated that the hippocampus is not required for the acquisition of stimulus-response habits (McDonald & White, 1993; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002) therefore if extinction was based on S-R associations, an impairment of latent extinction following hippocampal inactivation (Gabriele & Packard, 2006) would not be predicted. Further, since the dorsal striatum has been highly implicated in stimulus-response learning (Graybiel, 1998; McDonald & White, 1993; Packard & Knowlton, 2002; Knowlton et al., 1996; Packard, 2001; Packard, Hirsh, & White, 1989; White & McDonald, 2002) the lack of impairment in any form of extinction (including latent extinction) following caudate inactivation would be relatively unexpected. In sum, the present neuroanatomical double dissociation between latent and response extinction is inconsistent with the theory of a *single* mechanism that explains all extinction behavior.

An alternate explanation of latent extinction can be made in which un-reinforced goalbox placements cause the extinction of the Pavlovian association between the goalbox and reward which therefore reduce sign-tracking (Hearst & Jenkins, 1974) to the goalbox. However, this explanation is unlikely given several factors. Sign-tracking involves the approach to a localized discrete cue or stimulus; however learning situations in the context of diffuse spatial cues cause a generalized increase in activity rather than

specific sign tracking (Rescorla et al., 1985; Domjan, 2003). The current experimental parameters involve acquisition in an open maze with access to several extra-maze cues, none of which are in close proximity to the goalbox, therefore reducing the likelihood of explicit sign tracking. Similarly, hippocampal lesions do not affect sign-tracking behavior (Bussey et al., 2000; Parkinson et al., 2000; Ito et al., 2005). Moreover, given the dissociation of extinction behavior described above, the decrement in responding following latent extinction is not likely caused by reduced sign tracking, which would be expected to occur in both latent and response extinction. The present results suggest a reinterpretation of extinction theory to account for cognitive learning in which no S-R association is possible.

As seen in latent extinction, the inhibitory S-R theory of extinction has demonstrated some limitations in the difficulty explaining extinction under conditions when an explicit S-R association is not formed. However, another theory of extinction learning that encompasses some of these limitations is presented by Bouton (1996; 2004) in which extinction involves contextual modulation of either a CS-US or CS- noUS occasion setting. In this case, expectancy violations that are activated with non-reward initiate new learning about the CS that is context specific. Therefore a new inhibitory association is formed during extinction that allows the CS to have two potential associations with the US, the retrieval of which is based on context. Evidence for the contextual modulation of extinction is seen with the post-extinction relapse mechanisms of renewal and spontaneous recovery (Bouton & Swartzentruber, 1991; for review see Bouton 2002; 2004). The idea that extinction violates an expectancy and therefore leads to a contextually modulated CS-noUS association is more concurrent with latent

extinction, in which the animal learns that the location of the goalbox no longer predicts reward. However, according to a multiple memory systems hypothesis, it is possible that extinction involves both contextually modulated CS-noUS associations and inhibitory S-R associations depending on the task. It has clearly been demonstrated that inhibitory S-R association are formed during the extinction of some tasks (Rescorla, 1993a; 1993b; 1997), but not others (i.e. latent extinction). The current findings that demonstrate multiple forms of memory underlie extinction have implications for the involvement of multiple mechanisms to explain extinction behavior. Therefore, applying the multiple memory systems theory to extinction may allow for a better understanding of the neural bases of extinction behavior.

The Relative Use of Multiple Memory Systems During Extinction: Implications for Extinction of Drug Addiction

Evidence from the current experiments demonstrates that *requiring* a specific memory system during task acquisition can influence the relative effectiveness of multiple memory systems during extinction. Specifically, when learning is acquired solely through the caudate mediated stimulus-response habit system, cognitive extinction is ineffective. The idea that the way a task is learned can influence the way a task can be extinguished has many implications for clinical extinction therapies. These results show that in order for extinction therapies to be effective, the manner in which maladaptive behavior has been learned must be taken into account. However, while many human maladaptive behaviors involve a cognitive component, in some learning situations a transition from hippocampal based cognitive learning to striatal based stimulus-response habit learning occurs over extended training (McDonald & White, 1994; Packard &

McGaugh, 1996; Packard, 1999). Therefore, since acquiring a task with habitual S-R learning impairs cognitive extinction, the question arises whether cognitive forms of extinction would be affected once a transition to habitual learning occurs. Consistent with this hypothesis, previous studies have demonstrated an impairment in latent extinction following extended training in a straight alley (Dyal, 1963; Clifford, 1964).

This question has particular relevance to the extinction of drug addiction given that the switch from hippocampal dependent cognitive learning to caudate dependent habit learning switch potentially mirrors the switch from conscious goal-directed actions to the habitual drug seeking that may characterize addiction. Specifically, during drug seeking a transition may occur in a ventral to dorsal pattern within the striatum indicating that responding for drug reward becomes habitual over time (Everitt & Robbins, 2005; Haber et al., 2000; Porrino et al., 2004; See et al., 2007; Belin & Everitt, 2007). Additionally, theories of drug addiction propose that drug use subverts normal memory systems resulting in a more maladaptive form of habitual drug seeking (for review see Everitt et al., 2001). Since drug use may involve a maladaptive form of habitual learning and S-R habits are more difficult to extinguish (O'Keefe & Nadel, 1978; Osborne & Markgraf, 1988), cognitive behavioral therapies may be less effective. Correspondingly, the results from the present studies indicate that oral cocaine influences the relative effectiveness of multiple memory systems during extinction by impairing extinction under circumstances which new stimulus-response information is not available. While it remains to be seen whether this impairment could be due to an enhancement of the striatal memory system or an impairment of the hippocampal memory system, it appears that once learned behavior has transitioned to a strong habit, then cognitive behavior

therapies may be ineffective unless paired with some form of response extinction. For human drug addicts, cue exposure addiction treatments have been implemented, and while largely ineffective (Conklin & Tiffany, 2002), steps taken based on examining animal research (Conklin & Tiffany, 2002) to increase the effectiveness of this treatment may, in turn, increase the overall effectiveness of drug addiction treatment when combined with more cognitive forms of therapy. In summary, a multiple memory systems perspective may not only be beneficial but imperative for effective human extinction therapies. The multiple memory systems theory provides a framework with which to better investigate the potential drug-induced neurobiological changes, specifically in the hippocampal and dorsal striatal memory systems that may contribute to addiction in order to develop more successful enhancement of extinction processes.

Clinical Implications for the Pharmacological Enhancement of Latent Extinction

Further implications for the understanding of the neurobiological basis of latent extinction can be applied to human extinction therapies. For example, treatment of obsessive-compulsive disorder involves cognitive behavioral therapy, which consists of exposure coupled with response inhibition (for review see Marks, 1997). The specific prevention of normal rituals performs by those with OCD has obvious parallels with the latent extinction paradigm. The findings that the NMDA receptor agonism enhances latent extinction and hippocampal NMDA receptors are necessary for latent extinction to occur can direct further pharmacological treatments to be given in conjunction with cognitive behavioral therapy to enhance extinction. Further identifying the neurochemical basis of latent extinction can allow for the better development of pharmacological enhancement of cognitive forms of extinction.

Summary

Overall, the experiments described in this dissertation have demonstrated that, similar to initial acquisition, multiple memory systems are involved in extinction behavior through a double dissociation of latent and response extinction in a runway. These findings have distinct implications for the understanding of the neural bases of extinction indicating that there are multiple forms of extinction memory and that memory system employed during initial task acquisition can influence the relative use of multiple memory systems during extinction. Finally, the multiple memory systems theory provides a framework with which to further explore extinction behavior in order to develop more effective extinction therapies for the treatment of maladaptive behaviors.

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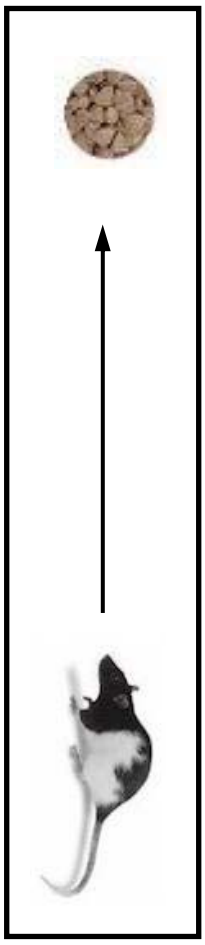
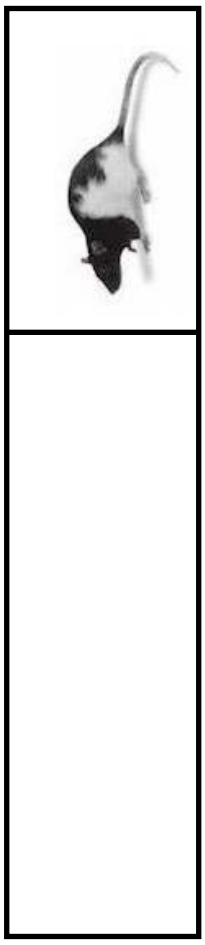
APPENDIX A

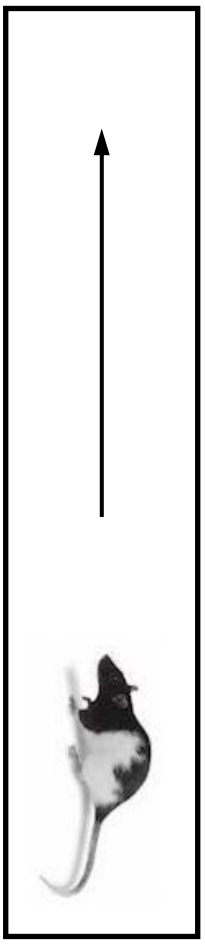
Latent Extinction



Acquisition

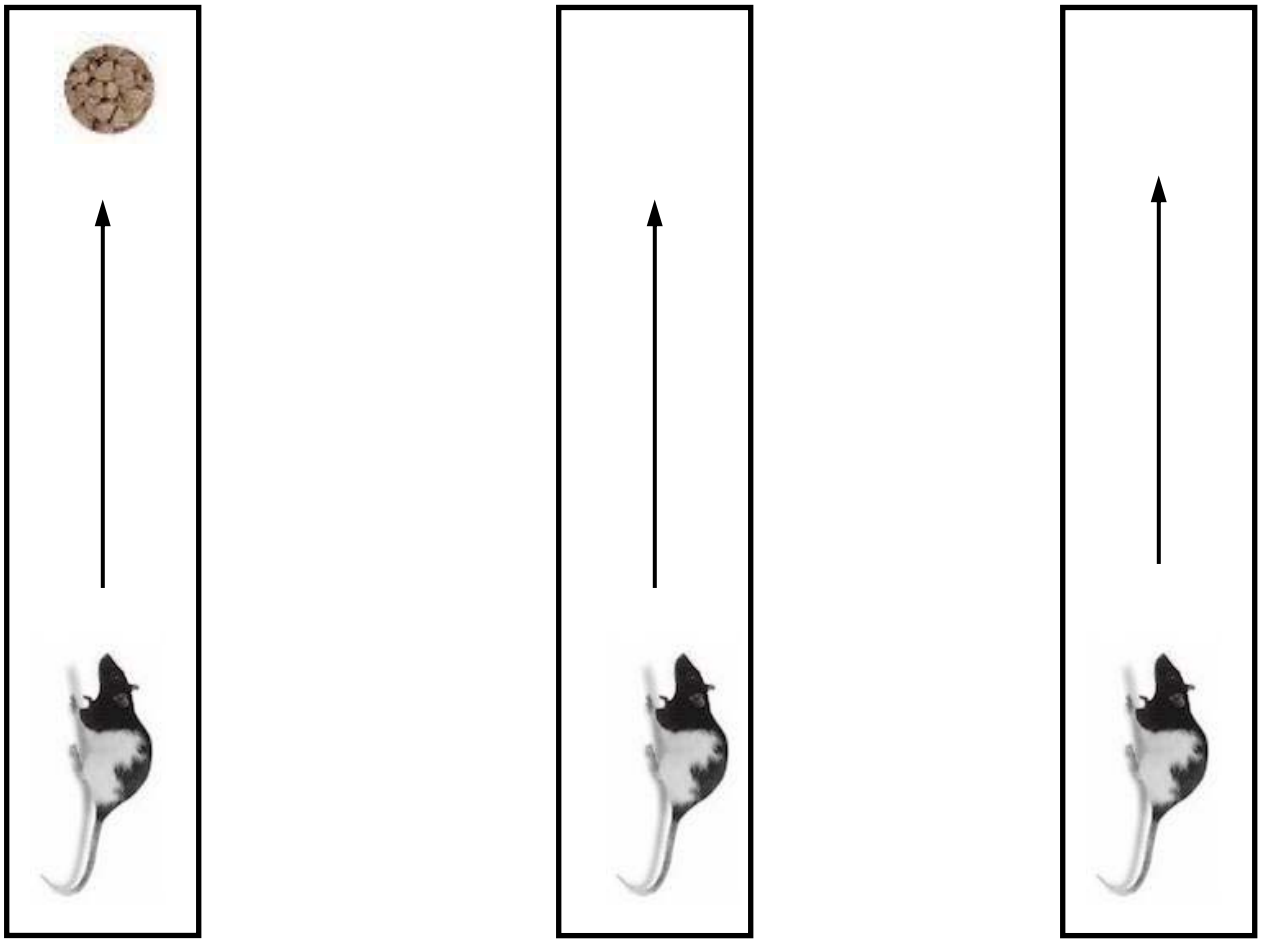


Latent Extinction



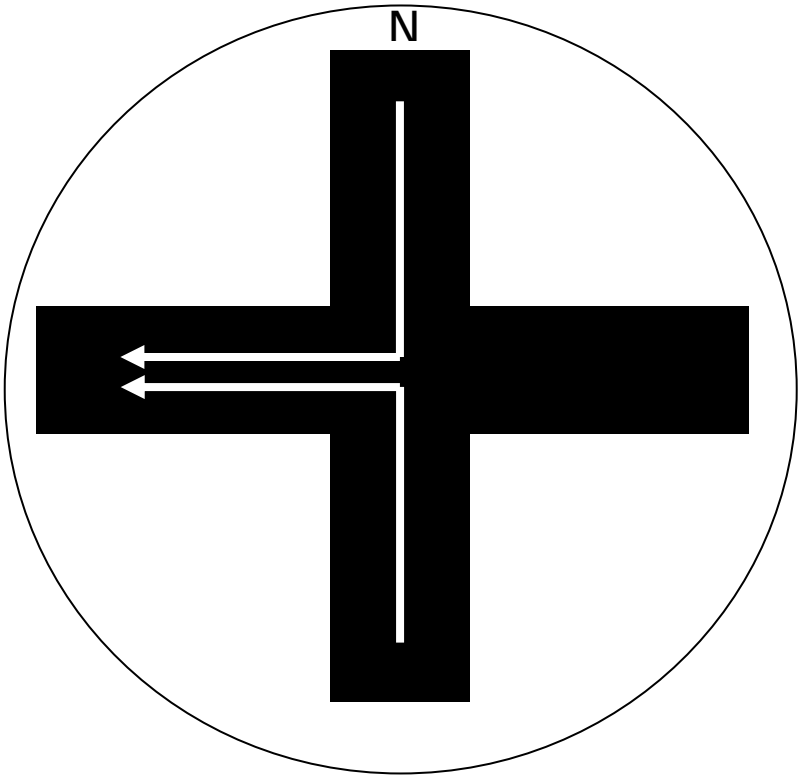
Probe Trials

Response Extinction

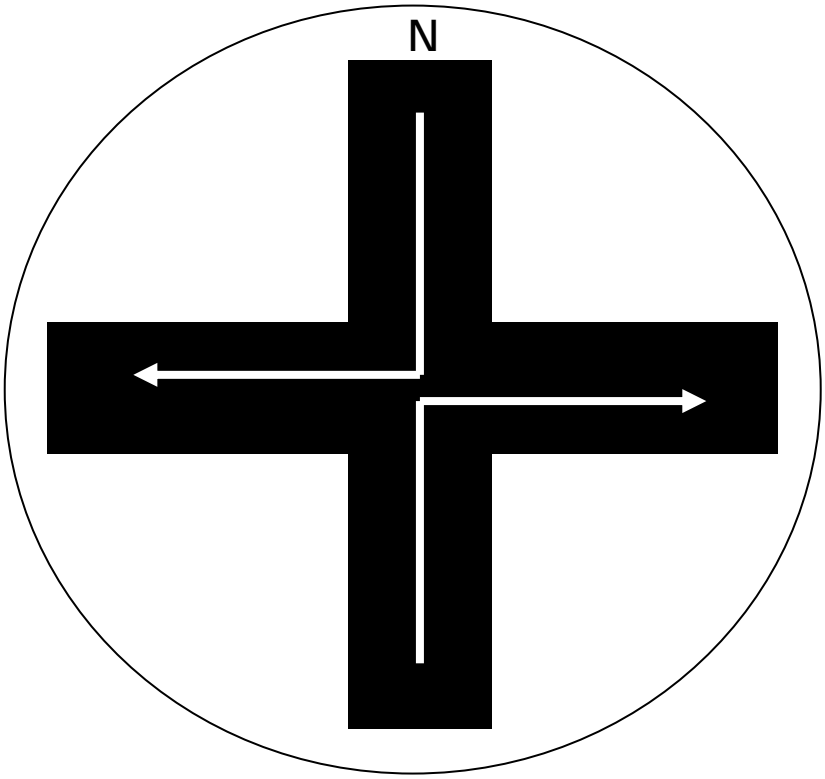


Acquisition Response Extinction Probe Trials

Single Solution Place Task

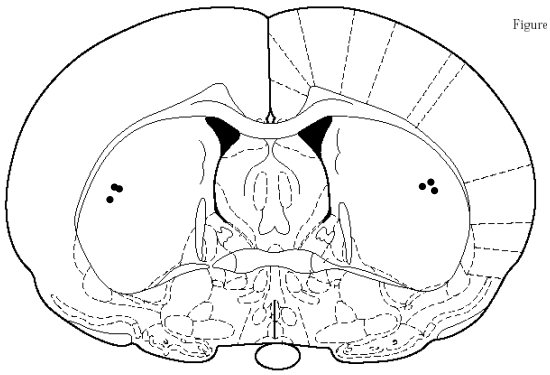


Single Solution Response Task



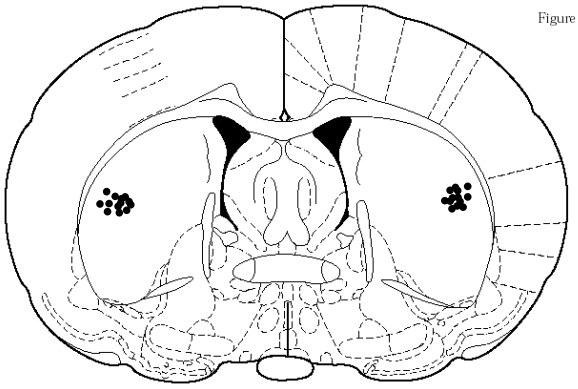
APPENDIX B

Figure 18



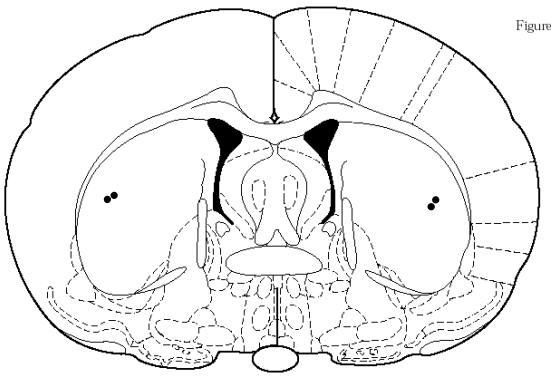
Interaural 8.74 mm Bregma -0.26 mm

Figure 19



Interaural 8.70 mm Bregma -0.30 mm

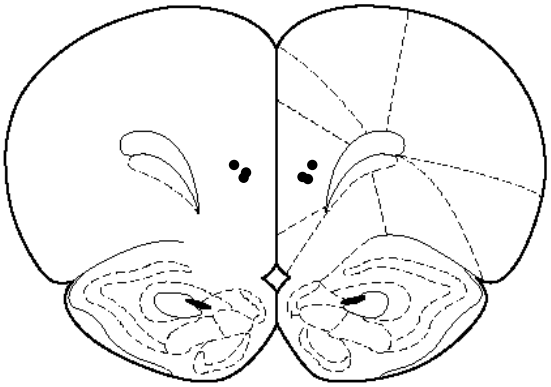
Figure 20



Interaural 8.60 mm Bregma -0.40 mm

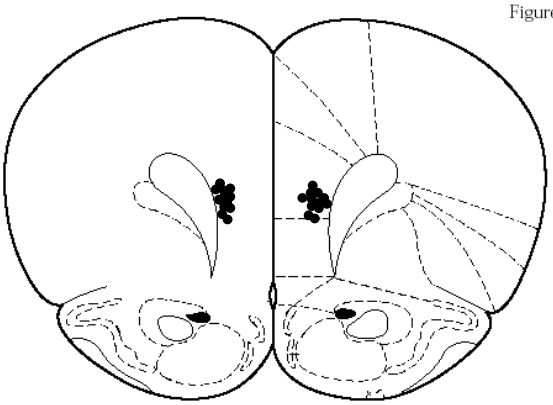
Coronal sections verifying dorsolateral caudate injection needle placement. The placements range from -0.26 (top) to -0.40 (bottom) anterior-posterior to bregma. (Adapted from Paxinos & Watson, 1997).

Figure 7



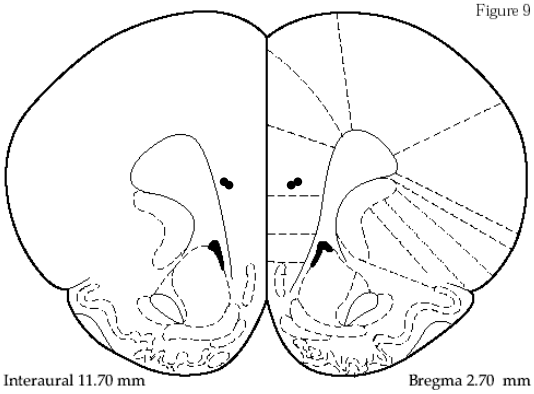
Interaural 12.70 mm Bregma 3.70 mm

Figure 8



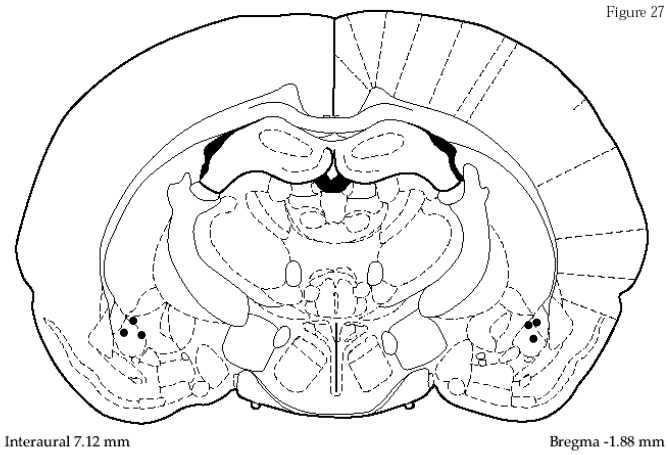
Interaural 12.20 mm Bregma 3.20 mm

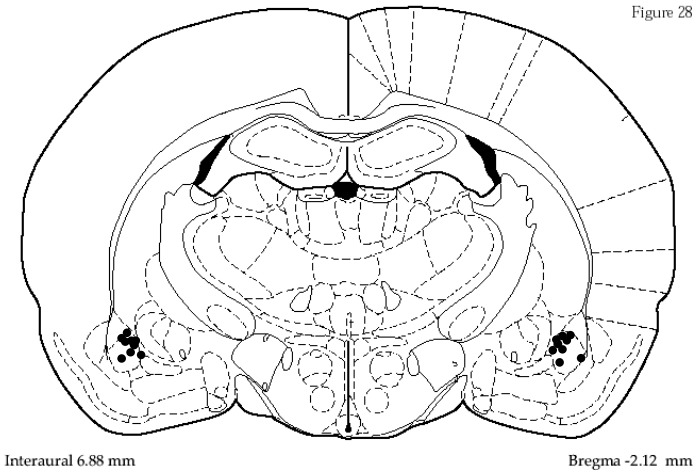
Figure 9

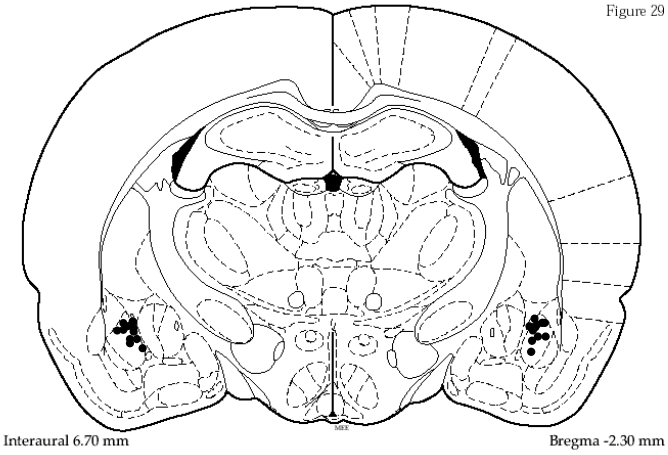


Interaural 11.70 mm Bregma 2.70 mm

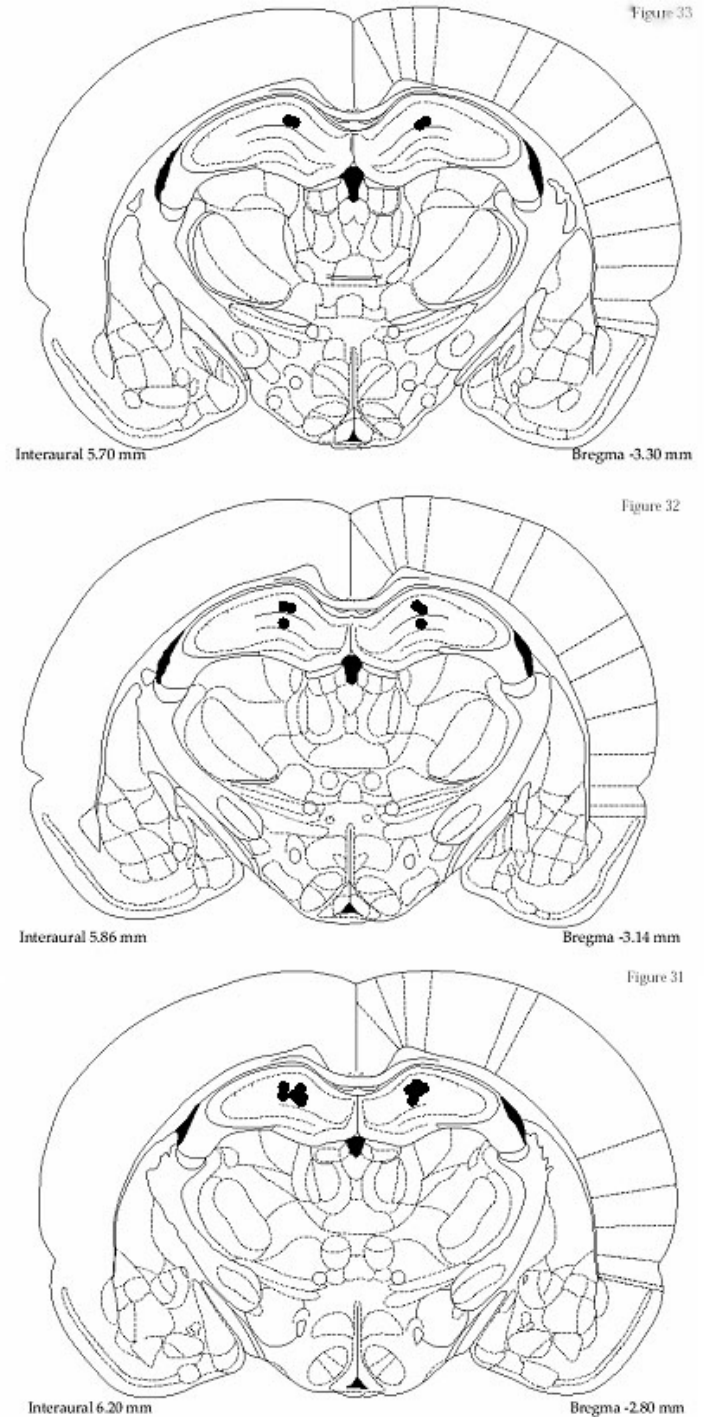
Coronal sections verifying medial prefrontal cortex injection needle placement. The placements range from +3.70 (top) to +2.70 (bottom) anterior-posterior to bregma. (Adapted from Paxinos & Watson, 1997).







Coronal sections verifying basolateral amygdala injection needle placement. The placements range from –1.88 (top) to -2.30 (bottom) anterior-posterior to bregma. (Adapted from Paxinos & Watson, 1997).



Coronal sections verifying dorsal hippocampus injection needle placement. The placements range from -3.30 (top) to -2.80 (bottom) anterior-posterior to bregma. (Adapted from Paxinos & Watson, 1997).

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PUBLICATIONS

Gabriele, A. & Packard, M.G. (2006). Evidence of a role for multiple memory systems in behavioral extinction. *Neurobiology of Learning and Memory, 85,* 289-299. ***Faculty of 1000, Recommended**

Gabriele, A. & Packard, M.G. (2007). D-cycloserine enhances memory consolidation of hippocampus-dependent latent extinction. *Learning and Memory, 14,* 468-471.

Gabriele, A. & Packard, M.G. (2008) Multiple memory systems and extinction: Requiring habit memory for task acquisition can render cognitive memory ineffective during extinction.*Manuscript in preparation.*

Gabriele, A., Setlow, B., & Packard, M.G. (2008). Oral cocaine administration selectively impairs latent extinction in a runway. *Manuscript in preparation.*

Gabriele, A. & Packard, M.G. (2008). Further evidence of a role for multiple memory systems in behavioral extinction. *Manuscript in preparation*.

Carlin, J., Gabriele, A. & Packard, M.G. (2008). Basolateral amygdala inactivation blocks enhancement of habit learning produced by peripheral anxiogenic drug administration. *Manuscript in preparation.*